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Box 42, Schermerhorn Hall, Columbia University, New York, N.Y.

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Organization and light relations in Polysphondylium

R. A. Harper

In my account of the processes by which the integration of the unicellular myxamoebae into pseudoplasmodia and sorocarps is brought about, I have given data as to the general range of their variation in form and size (Harper, 1929). In these studies considerable evidence was accumulated that *Polysphondylium* can develop and mature its sorocarps in either the ordinary alternation of daylight and darkness or in continuous darkness. Other students have noted this fact and it is also generally reported—Van Tieghem (1880), Brefeld (1869, 1884), Olive (1902), Potts (1902)—that both *Dictyostelium* and *Polysphondylium* are positively phototropic under ordinary conditions of one-sided illumination. I have confirmed this fact by repeated observations, though I have not so far undertaken quantitative studies of the effect of varying intensities or wave lengths of light on the degree and nature of the phototropic response. Plants like *Polysphondylium* in which the processes of growth and cell multiplication are separated from those of morphogenesis and differentiation are favorable for the further analysis of the specific effects of environmental stimulation on the various phases and stages of growth, integration, organogenesis and histogenesis and the development of the plant as a whole. The experiments which I shall here describe deal with the morphotic relations of light as shown in the size and form of plants grown in relatively continuous darkness as compared with the size and form of plants grown under ordinary conditions of the alternation of day and night in the laboratory. Potts (1902) has reported that plants of *Polysphondylium* grown in darkness are larger than those grown in light, and Stameroff (1897) finds that light has a differential effect on the vegetative and fertile hyphae of *Mucor*.

My intention in not at once comparing plants grown in continuous darkness with others grown in continuous light was to determine, as far as possible, the effects on growth and morphogenesis of relatively continuous darkness as compared with those obtained under the ordinary conditions of development of the plants in nature. The cultures were grown in ordinary dung decoction with 2 per cent agar in Petri dishes. The Petri dishes were inverted and stacked under two bell jars. In all but one of the experiments (no. II) the two bell jars were placed on a shelf about five feet from a south window. In experiment II the cultures grown in light were placed on a shelf much nearer the window. One of each pair of bell jars was covered with a black cloth and the other was left exposed to the alternating

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diffuse daylight and darkness of the laboratory. The room temperatures ranged from approximately 22° to 26° C. The cultures under the covered bell jar were exposed to daylight for a few minutes while being examined each day for general rate of development. The black cloth covering did not exclude all light. The object of the experiment was, as noted, to test the behavior of the plants under somewhat accentuated but similar conditions to those which might occur in nature as a result of chance in the distribution of the spores and their development in more or less shaded locations. No attempt was made to achieve an absolutely equivalent inoculation of each Petri dish. The spores for a given experiment were sown by picking up entire sorocarps from a single culture and dragging them across the agar, the point of the needle or forceps dipping deep enough to form a roughened streak in the agar surface. The first crop of sorocarps in such cultures is quite regularly formed along these streaks. This facilitates the counting of the plants, whorls per plant, branches per whorl, etc.

The first series was started April 14, 1929. It consisted of sixteen cultures, eight in alternating day and night, and eight in relatively continuous darkness. The Petri dishes were stacked, as noted above, under two bell jars which, with one exception, were placed side by side on a shelf about five feet from a south window. As further noted, one of the bell jars was covered with black cloth and the other left exposed to the diffuse light of the laboratory. One culture of the first series grown in the dark was accidentally broken on April 17, so that the final records for this series were taken from seven cultures. One culture in the corresponding series grown in alternating light and darkness failed to develop any plants, so that the final records for this lot also were taken from seven cultures. For brevity I shall speak of the two series as those in light and those in darkness respectively, and they are also indicated in the tables by the letters *L* and *D*. The number of cultures ranged from four to ten in each experiment, and the observations were continued from April 1929 to January 1930. The rate of development of the plants in all six of the experiments involving a total of 99 cultures was about the same.

The spores germinated, multiplied by division, and passed into an encapsuled and clumped stage within about twenty-four hours from the time they were sown. So far as I can find this encapsuled and clumped stage has not been hitherto described. I shall give figures and descriptions of it in a further paper. On the second day as a rule the creeping together (synallaxis) and the building of sorocarps (anallaxis) began. On the third day sorocarps were generally present in most of the cultures, and records for number of plants, number and order of whorls, and number of branches per whorl were generally taken from the fourth to the sixth day.

The number of sorocarps continues to increase for several days but the difficulty of getting accurate records also increases as the plants fall over and become tangled together. The sorocarps do not of course increase in size after they are once formed, so that a record of completed sorocarps taken within a few days of the time when they begin to form may be regarded as a fair average for the crop. It will be noted that the total number of plants recorded as having relatively large numbers of whorls (from 12 to 21) are from the later experiments. This is due in part at least to increasing skill in manipulation, whereby cultures with larger numbers of well distributed plants were produced, and to the resulting greater facility in making the counts of plants, whorls, and branches. The difficulty of following out the individual plants when they come to fall over and lie criss cross is considerable, and fewer of these larger plants were recorded from the earlier cultures. However the relative number of plants, whorls, and branches in the light and dark cultures respectively are fairly consistent. As shown in table 1 in each of the six series there is a smaller total number of plants and a smaller average number of plants per culture in the cultures grown in relative darkness than in the corresponding cultures grown in alternating day and night, and for the whorls and branches per plant a reverse tendency is shown. In each of the six series the higher average numbers of whorls per branched plant, the larger numbers of branches and the higher average numbers of branches per branched plant are found in the cultures grown in relative darkness. This holds true also whether, as in experiments II, III, IV, and VI (table 1), correction is made for the discrepancy of one in the number of cultures in light and darkness respectively. There is also a consistent excess in the number of unbranched plants in the light cultures as compared with those in darkness, with the single exception of experiment III.

As noted, the general type of erect radially and metamerically symmetrical organization and the method by which the integration and differentiation of the myxamoebae is brought about are described in a former paper (1929). The general plan of organization duplicates that of one of the higher cormophytes, except that there is no root system in *Polysphondylium*. Such plants grown in abundance in Petri dish cultures are favorable material for the quantitative study of the number and dimensions of the stipes, nodes and internodes, whorls of branches, terminal segments and sori, and the problems of symmetry of the plant as a whole. In the present paper I am presenting data as to the range in number of the whorls and branches of the sorocarp and the effect of light on the development of such structural elements.

TABLE 1

Summary of total cultures, plants, whorls, and branches

EXP.	CULT.		TOTAL PLANTS				RANGE				UNBRANCHED PLANTS				BRANCHED PLANTS	
					AV. PER CULT.		L		D		NUMBER		AV. PER CULT.		NUMBER	
	L	D	L	D	L	D	MIN.	MAX.	MIN.	MAX.	L	D	L	D	L	D
I	7	7	508	346	72.57	49.42	31	92	39	61	328	130	46.85	18.55	180	216
II	5	4	221	131	44.3	32.7	19	70	18	55	158	51	31.6	12.75	63	80
III	9	10	274	267	30.44	26.7	21	38	9	50	65	75	7.22	7.50	209	192
IV	9	9	354	204	39.33	22.66	17	76	4	39	80	31	8.88	3.44	274	173
V	10	10	887	317	88.7	31.7	13	190	12	67	260	45	26.00	4.5	627	272
VI	10	9	696	304	69.6	33.7	31	126	13	66	134	22	13.4	2.49	562	282
Totals	50	49	2940	1569	58.8	32.02					1025	354	20.5	7.22	1915	1215
Grand Totals			4509								1379				3130	

(*Continuation*)

EXP.	BRANCHED PLANTS		WHORLS				BRANCHES							
	AV. PER CULT.		NUMBER		AV. PER BR. PLANT		NUMBER		AV. PER BR PL.		AV. BR. PER WHORL		AV. BR. PER PL. FOR ALL PLANTS	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	25.71	30.85	363	863	2.01	3.99	864	2097	4.8	9.7	2.38	2.42	1.70	6.06
II	12.64	20.00	120	317	1.90	3.96	237	828	3.76	10.35	1.97	2.56	1.07	6.32
III	23.22	19.2	793	1028	3.79	5.35	2091	2595	10.00	13.51	2.62	2.52	7.63	9.71
IV	30.4	19.22	1183	1071	4.31	6.19	2391	2385	8.72	13.78	2.02	2.22	6.75	11.69
V	62.7	27.2	2372	1752	3.78	6.44	4916	3533	7.84	12.98	2.07	2.01	5.54	11.14
VI	56.2	31.3	2301	1935	4.09	6.86	4560	4126	8.11	14.63	1.98	2.13	6.55	13.57
Totals	38.3	24.79	7132	6966	3.72	5.73	15059	15564	7.86	12.80	2.11	2.23	5.12	9.91
Grand Totals			14098				30623							

The metameric segments of *Polysphondylium* may be compared structurally with such elements as the phytons (Gaudichaud, 1843; Velenovský, 1905) each consisting of a node, whorl of branches, and an internode of the stipe as in the organization of the higher plants. The range in number of such units which can be combined in a single plant and the relative numbers of plants with each possible number of phytons which will be found in a mixed population such as is produced in Petri dish cultures are shown in tables 1–4.

MORPHOGENESIS AND PHOTOMORPHOSIS

The total number of plants whose structural organization was recorded in the six experiments was 2940 in the light cultures, and 1569 grown in relative darkness, as shown from the summary in table 1. The range in total plants in the six experiments is from 221 plants (exp. II) to 887 plants (exp. V) in light, and from 131 plants (exp. II) to 317 plants (exp. V) in darkness. The average number of plants per culture for the six experiments is 58.8 in light to 32.0 in darkness. The range in the average number of plants per culture (table 1) for the plants in the cultures in alternating day and night was from 30.4 (exp. III) to 88.7 (exp. V). In darkness the range was from 22.6 (exp. IV) to 49.4 (exp. I). The range in total number of plants recorded per culture for the whole series in the light was from thirteen in culture 605 (exp. V) to 190 in culture 598 (exp. V). The range for the whole series of cultures in darkness was from four in culture 505 (exp. VI) to 67 in culture 612 (exp. V).

In my cultures of *Polysphondylium*, as noted in my former paper (1929) there was always a considerable number of unbranched plants (*Dictyostelium* type). So far as tested, cultures from these gave no different results than those from branched plants. The total number of unbranched plants in the six experiments was 1379. The total number of branched plants was 3130. Of the unbranched plants 1025 were produced in the light cultures to 354 in the dark cultures, an average per culture respectively of about twenty to seven. Of the branched plants 1915 were produced in the light cultures to 1215 in the dark cultures. The averages per culture were respectively about thirty-eight for the light and twenty-four for darkness.

In table 2 the plants are grouped according to the number of whorls of branches per plant, and the extreme range is from plants with a single whorl of branches, of which there are 521, 377 in the light cultures to 144 in the dark cultures, to plants with twenty-one whorls of branches, of which there are but two, one in a light culture and one in a dark culture. The numbers of plants in the groups between these extremes form a series with its high point (585) in the group with two whorls of branches, 419 in

TABLE 2

Plants grouped according to number of whorls per plant

EXP.	CULTURES		TOTAL PL.		NUMBER OF PLANTS PER GROUP															
					1		2		3		4		5		6		7		8		9	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	7	7	508	346	71	25	62	34	32	40	7	38	5	21	2	33	1	15	—	4	—	4
II	5	4	221	131	28	9	21	21	10	14	1	11	2	5	1	4	—	7	—	4	—	2
III	9	10	274	267	22	32	44	36	49	17	26	21	26	12	21	16	9	11	4	6	5	6
IV	9	9	354	204	40	21	51	21	48	17	39	11	28	20	17	7	14	15	5	11	8	11
V	10	10	887	317	127	30	128	24	114	21	77	30	53	32	40	18	26	13	10	19	14	21
VI	10	9	696	304	89	27	113	30	95	26	85	27	49	16	44	23	19	20	15	22	18	24
Totals	50	49	2940	1569	377	144	419	166	348	135	235	138	163	106	125	101	69	81	34	66	45	68
Grand Totals	99		4509		521		585		483		373		269		226		150		100		113	

(*Continuation*)

EXP.	10		11		12		13		14		15		16		17		18		19		20		21	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
II	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	1	6	—	8	—	4	2	4	—	4	—	3	—	4	—	—	—	—	—	—	—	1	—	1
IV	6	8	6	14	4	2	3	6	2	3	1	3	1	—	1	—	—	1	—	2	—	—	—	—
V	16	23	11	7	3	11	3	7	—	3	1	3	—	2	1	4	1	3	1	—	—	1	1	—
VI	10	10	5	12	4	7	5	6	4	9	3	4	2	5	—	6	2	4	—	2	—	2	—	—
Totals	33	49	22	43	11	25	13	23	6	19	5	13	3	11	2	10	3	8	1	4	—	4	1	1
Grand Totals	82		65		36		36		25		18		14		12		11		5		4		2	

the light cultures and 166 in the dark cultures. Only ninety-one out of 4509 plants have fourteen or more whorls each. Over one-third (1106) of the total of 3130 branched plants are in the groups with one and two whorls of branches. One-half (1565) of the total number of branched plants would not include all of the groups with one, two, and three whorls, which total 1589 plants. The average branched plant in the light cultures has about 3.72 whorls against 5.73 whorls per plant in the cultures in darkness (table 1). The average number of branches per whorl in the light and dark cultures are respectively 2.11 and 2.23 (table 1). Whether the plants as grown in inverted dung agar Petri dish cultures are more or less luxuriant than those occurring in nature in the soil and on dung must be determined by culturing them further under controlled conditions as to substratum, moisture, etc. From the largest single group with two whorls of branches the number of plants per group diminishes continuously with the increasing number of whorls except that the group with nine whorls (113 plants) is larger than the group with eight whorls (100 plants) and the groups with twelve and thirteen whorls of branches show the same number of plants, (36). For the first six groups the number of plants in the light cultures is consistently larger, but from the seventh group to the twenty-first group the situation is reversed, and the numbers of plants in the dark cultures taken by groups are consistently larger, except that in the group with twenty-one whorls of branches there is one plant each in both the light and dark culture. The influence of light in increasing the number of plants and diminishing their size, as indicated by the number of whorls of branches which they bear, is thus shown quite consistently. If the series were plotted the graphs would cross each other at the seventh group.

The total number of whorls in each group for the light and dark cultures is given in table 3. The largest number of whorls for a single group (1492) is found in the group with four whorls per plant. In the cultures grown in light the largest number of whorls is in the group with three whorls per plant (1044), and in the case of cultures grown in darkness the largest number of whorls (612) is in the group with nine whorls per plant.

In the cultures grown under conditions of alternating day and night as in the case of the plant numbers, the number of whorls per group is consistently higher up to the sixth group, and from there on the relation is reversed and the number of whorls per group is consistently higher in the cultures grown in relatively continuous darkness, except in the single case of the two plants with twenty-one whorls. The total number of plants produced in the light is upward of twice that produced in the dark (2940 to 1569) (table 1). The total number of whorls is larger in the plants grown in the light by only 1+ per cent (7132 in light to 6966 in darkness).

TABLE 3

Whorls grouped according to number of whorls per plant

EXP.	CULTURES		TOTAL WHORLS		NUMBER OF WHORLS PER GROUP															
					1		2		3		4		5		6		7		8		9	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	7	7	363	863	71	25	124	68	96	120	28	152	25	105	12	198	7	105	—	32	—	36
II	5	4	120	317	28	9	42	42	30	42	4	44	10	25	6	24	—	49	—	32	—	18
III	9	10	793	1028	22	32	88	72	147	51	104	84	130	60	126	96	63	77	32	48	45	54
IV	9	9	1183	1071	40	21	102	42	144	51	156	44	140	100	102	42	98	105	40	88	72	99
V	10	10	2372	1752	127	30	256	48	342	63	308	120	265	160	240	108	182	91	80	152	126	189
VI	10	9	2301	1935	89	27	226	60	285	78	340	108	245	80	264	138	133	140	120	176	162	216
Totals	50	49	7132	6966	377	144	838	332	1044	405	940	552	815	530	750	606	483	567	272	528	405	612
Grand Totals	99		14098		521		1170		1449		1492		1345		1356		1050		800		1017	

(*Continuation*)

EXP.	10		11		12		13		14		15		16		17		18		19		20		21	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	—	10	—	—	—	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
II	—	10	—	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	10	60	—	88	—	48	26	52	—	56	—	45	—	64	—	—	—	—	—	—	—	20	—	21
IV	60	80	66	154	48	24	39	78	28	42	15	45	16	—	17	—	—	18	—	38	—	—	—	—
V	160	230	121	77	36	132	39	91	—	42	15	45	—	32	17	68	18	54	19	—	—	20	21	—
VI	100	100	55	132	48	84	65	78	56	126	45	60	32	80	—	102	36	72	—	38	—	40	—	—
Totals	330	490	242	473	132	300	169	299	84	266	75	195	48	176	34	170	54	144	19	76	—	80	21	21
Grand Totals	820		715		432		468		350		270		224		204		198		95		80		42	

The total number of branches produced in the light and dark cultures in the six experiments and their distribution in the groups based on whorls per plant are given in table 4. A total of 30623 branches were produced and they were distributed about equally between the light and dark cultures (15059, 49.1 per cent, in light to 15564, 50.8 per cent in darkness). The largest number of branches (3467) in any one group is found in that with three whorls per plant. The general trend of the series is similar to that for the numbers of plants and for the numbers of whorls as shown in tables 2 and 3. The largest number of branches in any one group of the light cultures (2474) is also in the group with three whorls of branches. The largest number of branches in the dark cultures (1412) is in the group of plants with six whorls per plant. The number of branches per group is higher in the light cultures than in the dark cultures in the groups with from one to six whorls of branches. In the plants with from seven to twenty-one whorls there are consistently more branches in the dark cultures. There is a consistent excess in the dark cultures from the seventh to the twenty-first group inclusive. The single plant with twenty-one whorls of branches in the dark cultures has forty-eight branches against forty-three branches, for the corresponding single plant with twenty-one whorls in the light cultures. The average number of branches per whorl for all the branched plants studied, as noted above, is 2.11 for the light cultures and 2.23 for the dark cultures (table 1).

Table 5 shows the average number of branches per whorl for each of the six experiments in the successive groups of plants with from one to twenty-one whorls of branches per plant. There is fluctuation in the average number of branches per whorl both in the experiments taken separately and in the relations of the results in the light and dark cultures. The averages for all the groups from one to twenty-one is consistently higher for the cultures grown in relatively continuous darkness. The highest average number of branches per whorl (2.4) is in the group of plants grown in darkness with three whorls of branches per plant. The averages for the five dark grown groups with from two to six whorls are 2.3 or more. The last five groups grown in darkness have averages of about two, except the single plant with twenty-one whorls, which has an average of 2.2 branches per whorl.

In the series of groups grown in alternating day and night the first eight groups have averages of from 2.04 to 2.36 branches per whorl. The highest average 2.36 is, as in the case of the dark grown plants, in the group with three whorls of branches per plant. The remainder of the light cultures in the groups with from nine to twenty-one whorls per plant have

TABLE 4

Branches grouped according to number of whorls per plant

EXP.	CULTURES		TOTAL BRANCHES		NUMBER OF BRANCHES PER GROUP															
					1		2				4			6		7		8		9	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	7	7	864	2097	163	57	307	162	238	321	58	398	61	258	24	473	13	242	—	71	—	72
II	5	4	237	828	57	20	79	102	68	115	5	104	22	65	6	59	—	121	—	101	—	55
III	9	10	2091	2595	55	79	217	181	417	130	284	229	313	150	369	289	151	169	89	136	111	130
IV	9	9	2391	2385	83	49	225	103	334	127	355	110	294	257	199	89	194	215	77	192	128	219
V	10	10	4916	3533	249	55	564	103	764	136	647	243	578	329	507	209	393	182	172	317	236	408
VI	10	9	4560	4126	170	65	483	131	653	164	697	215	514	160	544	293	262	295	219	337	302	445
Totals	50	49	15059	15564	777	325	1875	782	2474	993	2046	1299	1782	1219	1649	1412	1013	1224	557	1154	777	1329
Grand Totals	99		30623		1102		2657		3467		3345		3001		3061		2237		1711		2106	

(*Continuation*)

EXP.	10		11		12		13		1		15		16		17		18		19		20		21	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	—	22	—	—	—	21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
II	—	25	—	61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	27	150	—	236	—	125	58	120	—	129	—	105	—	155	—	—	—	—	—	—	—	34	—	48
IV	108	155	130	321	93	57	64	181	46	85	16	111	26	—	19	—	—	40	—	74	—	—	—	—
V	290	487	225	172	57	255	63	186	—	75	18	74	—	50	37	118	41	94	32	—	—	40	43	—
VI	175	228	86	290	77	176	95	164	92	285	73	145	53	167	—	233	55	167	—	80	—	86	—	—
Totals	600	1067	441	1080	227	634	280	651	138	574	107	435	79	372	56	351	96	301	32	154	—	160	43	48
Grand Totals	1667		1521		861		931		712		542		451		407		397		186		160		91	

averages of from 1.42 to 1.95, except in the case of the single plant with twenty-one whorls, whose average is 2.04. There is a tendency for the groups with from one to six whorls of branches to have higher average numbers of branches per whorl. This difference is slightly greater in the cultures grown in alternating day and night, than in those grown in relatively continuous darkness. The possible significance of such data may become more clear in the light of extensive measurements of length of branches, diameters of sori, etc.

For a more detailed study of these light effects we may turn again to the summary given in table 1, where, as also in tables 2–9. the numbers are all based directly on the original data as given by the 50 cultures in light and the 49 cultures in relative darkness. I am noting in the text in certain cases the results given when allowance is made for the difference of one in the number of light and dark cultures in experiments II, III, VI, and in the totals. These differences affect only the evidence as to the degree of the light effects.

As noted, for all six experiments the total number of plants in the cultures grown in alternating daylight and darkness is 2940 as compared with 1569 for the plants grown in relatively continuous darkness. There is an excess of 1371 in the number of plants in favor of the light cultures. In each experiment taken separately the total number of plants produced in the light was greater than that in the darkness.

In experiment III there was one more Petri dish culture in the dark series than in the light series. In experiments II and IV there was one more culture in the light series than in the dark series. The total number of cultures in the light was 50 against 49 in the dark. Allowing for the additional culture in the light, the totals become 2882 plants produced in light against 1569 plants produced in the dark, an excess of 1312 in favor of the light. That is equal numbers of cultures in the light and in the dark produced respectively 64 per cent and 35 per cent of the total number of plants.

The total number of unbranched plants produced in the six experiments was 1379, of which 1025 (74%) were in the light cultures and only 354 (25%) in those grown in relative darkness. There is an excess of unbranched plants in the light cultures of every experiment except the third in which there were 9 cultures in the light and 10 in darkness. If we allow for the difference of one additional culture in the darkness in experiment III the number of unbranched plants becomes 67.5 for the dark cultures, which is 2.5 more than the number in the corresponding cultures grown in light (65). As noted there is also an excess of one each in the number of cultures in light in experiments II and VI. Corrections for the differences

TABLE 5

Average branches per whorl grouped according to number of whorls per plant

EXP.	GRAND AVERAGES		1		2		3		4		5		6		7		8		9		10	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	2.3	2.4	2.2	2.2	2.4	2.3	2.4	2.6	2.0	2.6	2.4	2.4	2.0	2.3	1.8	2.3	—	2.2	—	2.0	—	2.2
II	1.9	2.6	2.0	2.2	1.8	2.4	2.2	2.7	1.2	2.3	2.2	2.6	1.0	2.4	—	2.4	—	3.1	—	3.0	—	2.5
III	2.6	2.5	2.5	2.4	2.4	2.5	2.8	2.5	2.7	2.7	2.4	2.5	2.9	3.0	2.3	2.1	2.7	2.8	2.4	2.4	2.7	2.5
IV	2.0	2.2	2.0	2.3	2.2	2.4	2.3	2.4	2.2	2.5	2.1	2.5	1.9	2.1	1.9	2.0	1.9	2.1	1.7	2.2	1.8	1.9
V	2.0	2.0	1.9	1.8	2.2	2.1	2.2	2.1	2.1	2.0	2.1	2.0	2.1	1.9	2.1	2.0	2.1	2.0	1.8	2.1	1.8	2.1
VI	1.9	2.1	1.9	2.4	2.1	2.1	2.2	2.1	2.0	1.9	2.0	2.0	2.0	2.1	1.9	2.1	1.8	1.9	1.8	2.0	1.7	2.2
Averages			2.0	2.2	2.2	2.3	2.3	2.4	2.1	2.3	2.1	2.3	2.1	2.3	2.0	2.1	2.0	2.1	1.9	2.1	1.8	2.1

(*Continuation*)

EXP.	11		12		13		14		15		16		17		18		19		20		21	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
I	—	—	—	1.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
II	—	2.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	—	2.6	—	2.6	2.2	2.3	—	2.3	—	2.3	—	2.4	—	—	—	—	—	—	—	1.7	—	2.2
IV	1.9	2.0	1.9	2.3	1.6	2.3	1.6	2.0	1.0	2.4	1.6	—	1.1	—	—	2.2	—	1.9	—	—	—	—
V	1.8	2.2	1.5	1.9	1.6	2.0	—	1.7	1.2	1.6	—	1.5	2.1	1.7	2.2	1.7	1.6	—	—	2.0	2.0	—
VI	1.5	2.1	1.6	2.0	1.4	2.1	1.6	2.2	1.6	2.4	1.6	2.0	—	2.2	1.5	2.3	—	2.1	—	2.1	—	—
Averages	1.8	2.2	1.7	2.1	1.6	2.1	1.6	2.1	1.4	2.2	1.6	2.1	1.6	2.0	1.7	2.0	1.6	2.0	—	2.0	2.0	2.2

in these cases, however, still leave a notable excess in the number of plants in the light cultures.

The average number of unbranched plants per culture in the light series in the data recorded for experiment III was 7.2. In the dark cultures it was 7.5, only a fractional difference in favor of the dark cultures. In the remainder of the experiments the average excess of unbranched plants per culture in the light ranged from 5.4 in experiment IV to 28.3 in experiment I.

The total number of branched plants produced in the six experiments was 1915 in the light and 1215 in the darkness, a total of 3130 (table 1). While there is an excess of branched plants in the light cultures it is by no means so conspicuous as in the case of the unbranched plants. The average numbers of branched plants per culture in light and darkness respectively are 38 in light to 24 in darkness, as compared with 20 in light to 7 in darkness for the unbranched plants. As shown in table I, the average number of branched and unbranched plants per culture taken together is consistently higher in the light in all six experiments. The averages for the total plants in the six experiments are 58 plants in light to 32 plants in darkness.

Allowing for the additional culture in the light the totals are 1004 unbranched plants in light to 354 unbranched plants in darkness. That is 73 per cent of the unbranched plants were produced in the light cultures, against 26 per cent produced in darkness. In the case of the branched plants the difference in their distribution between dark and light cultures is not so marked and is much more irregular. There is an excess of branched plants in the light cultures in four of the experiments, numbers III to VI, while in experiments I and II there is an excess of branched plants in the dark cultures. Allowing for the additional culture in the light series the totals for the branched plants are 1876.7 in the light cultures and 1215 in the dark cultures. That is about 60 per cent of the branched plants appeared in the light cultures to 39 per cent in the dark cultures a difference by no means so marked as in the case of the unbranched plants. It is apparent that for both unbranched and branched plants the ordinary alternation of day and night tends to increase the number of plants per culture as compared with relatively continuous darkness.

Table I also shows the total numbers of whorls and branches produced in the 99 cultures and the average number of whorls per unbranched and branched plant in light and darkness. Taking account of the additional culture in the light series the total number of whorls for the plants grown in light is 6989.3 against 6966 for the plants in darkness, a difference of about twenty-three. That is, of the 13956 whorls produced by 98 cultures, 50 per cent were borne by the plants in the light cultures to 49 per cent

in the dark cultures. As the table shows, the average number of whorls per branched plant is consistently higher in each experiment for the plants grown in relative darkness. For all six experiments the average number of whorls per branched plant in the light is 3.72 and in darkness 5.73.

The total numbers of branches in the light and dark cultures, are respectively 15059 and 15564, an excess of 1.7 per cent for the plants grown in darkness. Allowing for the extra culture in the light series the totals become about 14758 and 15564 a grand total of about 30322 branches for 98 cultures of which about 48 per cent were produced in the light cultures and 51 per cent in darkness.

The sori can be regarded as forming two groups: (*a*) the terminal sori, of which of course there is one for each sorocarp whether branched or unbranched, and (*b*) the lateral, which are the same in number as the branches. Of the total sori in the 99 cultures of both groups *a* and *b* (35132) there are 51.2 per cent from the light cultures and 48.7 per cent from the dark cultures, an excess of 2.5 per cent in favor of the light cultures. Making a reduction of one culture from the light series, the totals of apical sori become about 2882 in light and 1569 in darkness and making similar allowance for the extra culture in light in the case of the branches, we have totals of lateral sori of about 14758 in light and 15564 in darkness. The totals for both apical and lateral sori in light and darkness are respectively about 17640 (50.7%) in the light cultures and 17133 (49.2%) for the cultures in darkness—a difference of 1.5 per cent in favor of the cultures in light. The problem as to the relative number of spores produced in the light and dark cultures will of course require careful measurements of the size of the sori, etc. The apical sori are regularly larger than those on the branches and as noted my cultures show that relatively more unbranched sorocarps are produced in the light cultures.

The last two columns in table I give the average numbers of branches per plant (5.12 in light, 9.91 in darkness) for all plants including both branched and unbranched plants. This treatment of the unbranched plants as for the most part simply the result of light inhibition, makes more evident the increased proportion of branches produced in darkness. There is a consistent excess in the average number of whorls per branched plant and the average number of branches per plant in the dark cultures in all experiments. This is also true in the average number of branches per plant for all plants both branched and unbranched (table 1) and this latter difference is perhaps a more accurate measure of the influence of light on branching under the conditions prevailing in these experiments. However, it remains to be shown to what degree unbranched plants would disappear from cultures grown in total and continuous darkness.

The data in table 1 indicate that the normal alternation of day and night as contrasted with relatively continuous darkness tends to decrease the average number of whorls and branches per branched plant and increase the number of plants per culture so that the total numbers of whorls, branches and sori are not very different in the light and dark cultures. The average number of branches per whorl for all the branched plants is 2.17. For the branched plants in the light the average is 2.11; for those in the dark it is 2.23 (table 1).

Summarizing the data in table 1 and making an allowance for the additional culture in the light series it is shown that:

49 cultures in alternating day and night produced about:	The 49 cultures in relative darkness produced about:
65% of all the plants	35% of all the plants
(a) 73% of the unbranched	(a) 26% of unbranched
(b) 60% of the branched	(b) 39% of branched
50% of the whorls	49% of the whorls
48% of the branches	51% of the branches
50% of the total sori	49% of the total sori

The excess of whorls, branches and sori per plant computed for the average plant may be summarized from the data in table 1 as follows. The average plant from the 49 dark cultures had:

(1) 21+% more whorls per average branched plant,
(2) 23+% more branches in the average branched plant, 31+% more branches in the average of all plants, both branched and unbranched,
(3) 28+% more sori per average plant,

than the average plant of the 50 light cultures.

The notable difference in the number of whorls, branches, and hence sori in the average plant from the cultures grown in alternating daylight and darkness, when compared with the average plant from the cultures grown in relatively continuous darkness while the total numbers of whorls, branches, and sori are very much the same for the light and dark cultures, brings out the relation of light to the size of plants as contrasted with number of plants which are formed from a given number of germ cells, and thus differentiates the light relations of growth and cell multiplication on the one hand and architectonic morphogenesis on the other.

The nature of the organization of the sorocarp and the effect of light upon it are further illustrated by classing:

(1) The plants in groups according to the number of branches per plant (table 6A).

(2) The whorls in groups according to the number of branches per whorl (table 6B).

TABLE 6A. *Plants grouped according to the number of branches per plant*

NO. BR.	1		2		3		4		5		6	
	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	110	34	187	70	159	59	179	68	171	67	176	63
Per cent	76	23	72	27	72	27	72	27	71	28	73	26

(*Continuation*)

NO. BR.	7		8		9		10		11		12	
	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	126	68	130	58	114	68	69	54	67	55	75	51
Per cent	64	35	69	30	62	37	56	43	54	45	59	40

(*Continuation*)

NO. BR.	13		14		15		16		17		18	
	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	61	38	44	41	46	34	31	43	32	40	20	31
Per cent	61	38	51	48	57	42	41	58	44	55	39	60

(*Continuation*)

NO. BR.	19		20		21		22		23		24	
	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	24	20	23	28	15	15	8	26	11	26	9	15
Per cent	54	45	45	54	50	50	23	76	29	70	37	62

(*Continuation*)

NO. BR.	25		26		27		28		29		30	
	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	7	11	4	12	3	21	3	10	1	9	2	11
Per cent	38	61	25	75	12	87	23	76	10	90	15	84

(*Continuation*)

NO. BR.	31		32		33		34		35		36		37	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	2	8	1	5	1	12	1	4	—	2	—	3	1	6
Per cent	20	80	16	83	7	92	20	80	—	100	—	100	14	85

(*Continuation*)

NO. BR.	38		39		40		41		42		43		44	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	—	4	—	—	—	5	1	1	—	4	1	3	—	3
Per cent	—	100	—	—	—	100	50	50	—	100	25	75	—	100

(*Continuation*)

NO. BR.	45		47		48		49		51		54		56	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D
No. Pl.	—	2	—	1	—	2	—	1	—	1	—	1	—	1
Per cent	—	100	—	100	—	100	—	100	—	100	—	100	—	100

As shown in table 6A, the number of branches per plant ranges from one to fifty-six. The group with two branches has the largest number of plants (257). This group also has the highest number of plants (187) in the light cultures. The highest percentage of plants is found in the group with only one branch per plant, 76 per cent in the light and 23 per cent in the dark cultures.

The percentage relations of the numbers of plants in the light and dark cultures when classed by the number of branches per plant show a striking uniformity in the first six groups, that is, the groups with respectively from one to six branches per plant. From the sixth group on the percentage of plants in the light tends to diminish but is in excess of the plants in darkness till the sixteenth group is reached, where the dark plants become relatively more numerous. No plants with more than forty-three branches appeared in the light cultures, and of these there was but one. This plant was one of three in the light cultures which had more than thirty-four branches, while in the dark cultures forty plants had more then thirty-four branches each. The largest number of branches in a single plant was, as noted, fifty-six. One plant each had forty-nine, fifty-one, fifty-four, and fifty-six branches, as shown in the table. The data in table 6A illustrate again the appearance of large numbers of plants with very few branches in the cultures as I have grown them, and must not necessarily be regarded as typical of the plants as they occur in nature.

There is a conspicuous excess of plants in the light cultures which extends to the group with fifteen branches, in which the numbers are forty-six plants in light to thirty-four in the dark cultures. From this point on the larger numbers of plants are consistently in the dark cultures, with two exceptions—the group with nineteen branches, which consists of twenty-four plants in the light to twenty in the dark and the group with twenty-one branches, in which the numbers of plants in the light and dark cultures are equal (fifteen).

The transition from the one distribution to the other comes in the region of the groups with from fifteen to twenty branches which may be taken in such a classification as the region of the typical plant, as grown in inverted Petri dish cultures, with five or six whorls of about three branches each,—possibly two branches in the first whorl, four branches in the apical whorl, and three or four intermediate whorls of three branches each. If the light is increased there will be a tendency to diminish the number of whorls and branches per plant. If the light is diminished there will be a tendency to increase the number of whorls and branches per plant. The average number of branches per plant in such a branched type is 7.8+ in light and 12.8+ in darkness (table 1).

One half of the total number of branched plants (1515) is included in the groups having from one to about seven branches per plant. One-half the total number of branches (15311) is found in the groups having from one to about six whorls of branches per plant (table 4). Including the light cultures of the group with six whorls gives 15221 branches.

When the branches are classed according to the number per whorl (table 6B) the group with a single branch per whorl is the largest (4512, thirty-two per cent) but the variation is not great till we pass the group with three branches per whorl, which totals for the light and dark cultures together 3960 (twenty-eight per cent). The group with four branches per whorl includes only nine per cent of the total. The groups with five and six branches per whorl each include less than one per cent of the total number of whorls. There are but six cases of whorls with six branches each. It is to be remembered here also that, as stated, the branches in such whorls are very likely not to be at exactly the same level on the stipe. In many cases the plants in such a group might almost better be classed as having whorls of four or five branches with an accessory branch or whorl of one branch standing slightly above or below the whorl. The distribution of the classes based on number of branches per whorl illustrates very well the nature of mixed classifications in which the individual cases in a series are determined by very different factors.

The whorl of three branches is perhaps to be regarded as the normal type, giving a compact arrangement of the cleavage sectors of the segmental mass as it divides. However, the fact that, as shown in table 6B, whorls with one, two, and three branches each tend to be fairly equal in number indicates that no very fundamental physical principle determines the cleavage of the segmental mass, nor perhaps its size as it abstricts itself from the sorogenic mass. Measurements of the length of the branches and the diameter of the sori in their mutual inter-relations and in their relation to the number of branches in the whorl may throw more light on the questions here involved.

The proportion of whorls of plants having respectively one and two branches each (32%, 29%) is about the same as the proportion of un-

TABLE 6B

The whorls grouped according to the number of branches per whorl

NO. OF BR.	1		2		3		4		5		6	
	L	D	L	D	L	D	L	D	L	D	L	D
	2390	2122	2203	2009	1948	2012	540	431	49	88	2	4
Total	4512		4212		3960		1271		137		6	
Per cent	32		29		28		9		.9		—	

branched plants (30%). When the plants are classed according to their number of branches the largest single group is, as noted, that with only two branches per plant as shown in table 6A.

METAMERISM AND TAPER

The evidence as to variation in the number of branches per whorl, when considered from the standpoint of the groups of plants based on the number of whorls per plant, has been given above and the tendency to higher numbers of branches per whorl in the groups of plants with smaller numbers of whorls per plant (6–10; table 5) was noted. These data do not, however, show whether in the individual plant such variations in the numbers of branches per whorl tend to be so distributed from base to apex of the plant as to result in a metameric taper. The figures of *Polysphondylium* commonly given in books of reference, and based on those of Olive and Brefeld, show prounounced taper from base to apex. As bearing on this question, the plants with three or more whorls were taken as a group which show basal and apical whorls and at least one intermediate whorl in which the number of branches per whorl can be compared. The distribution of basal, intermediate, and terminal whorls and branches is given in table 7. The plants with one or two whorls per plant were classed separately as a group in which metamerism is so slightly in evidence that a tendency to tapering could hardly come to expression.

The size of the nodal mass as abstricted from the sorogenic mass is doubtless a factor in determining the number of branches in a whorl, as noted, but the relation of length of branch and size of sorus to number of branches per whorl must be taken into account. Assuming that the segments abstricted are about the same in length, the size of any one of them will depend on the diameter of the sorogenic mass. If for any reason the sorogenic mass becomes more elongated and thinner the size of the nodal masses will be less and the number of branches per whorl will probably tend to be smaller. It is possible with suitable culture apparatus to grow the plants under conditions which will make it possible to photograph them at each successive stage in their development so that length and diameter of any nodal segment, and number and dimensions of each branch and sorus formed from it can be more or less accurately determined.

By varying the light, temperature, moisture, and other conditions it will be possible to determine whether and to what degree these dimensional relations can be modified by environmental changes. Studies bearing on such data are under way and I shall hope to report the results later. At present we are concerned with the evidence as to taper in sorocarps grown under the conditions above described.

The relative number of branches per whorl from base to apex of the sorocarp was determined for a group consisting of 2024 plants, each having as noted, three or more whorls per plant (table 7). The whorls were grouped according to their position as basal, intermediate, and terminal. The data as given show that the average number of branches in the apical and basal whorls of the plants in both light and dark cultures is slightly different than for the intermediate whorls. The average number of branches in the terminal whorl of all plants having three or more whorls of branches is 2.44 in the light and 2.45 in darkness. The average number for the basal whorl is 1.71 in the light and 1.69 in darkness, and for the intermediate whorls it is 2.10 in the light and 2.28 in darkness. There is thus shown perhaps a slight tendency for the top whorl to have more branches and the

TABLE 7

Distribution of whorls and branches in plants with three or more whorls

TOTALS

EXP.	NO. OF PLANTS		BASAL BRANCHES		INTERMEDIATE WHORLS		INTERMEDIATE BRANCHES		TERMINAL BRANCHES	
	L	D	L	D	L	D	L	D	L	D
I	47	157	98	324	74	456	180	1163	116	391
II	14	50	28	103	22	166	44	468	29	135
III	143	124	326	235	397	676	1103	1771	390	329
IV	183	131	283	210	675	746	1361	1688	439	335
V	372	218	596	316	1245	1238	2616	2569	891	490
VI	360	225	588	347	1266	1398	2450	3039	869	544
Totals	1119	905	1919	1535	3679	4680	7754	10698	2734	2224
Grand Totals	2024		3454		8359		18452		4958	

(*Continuation*)

AVERAGES

EXP	BASAL		INTERMEDIATE		TERMINAL		WHLS. PER PL. FOR BR. PLS.		BRS. PER WHORL	
	L	D	L	D	L	D	L	D	L	D
I	2.0	2.0	2.4	2.5	2.4	2.4	2.0	3.9	2.3	2.4
II	2.0	2.0	2.0	2.8	2.0	2.7	1.9	3.9	1.9	2.5
III	2.2	1.8	2.7	2.6	2.7	2.6	3.7	5.3	2.6	2.5
IV	1.5	1.6	2.0	2.2	2.3	2.5	4.3	6.1	2.0	2.2
V	1.6	1.4	2.1	2.0	2.3	2.2	3.7	6.4	2.0	2.0
VI	1.6	1.5	1.9	2.1	2.4	2.4	4.0	6.8	1.9	2.1
Average	1.71	1.69	2.10	2.28	2.44	2.45	3.7	5.7	2.1	2.2

basal whorl to have fewer branches than do the intermediate whorls in plants with three or more whorls. Table 7 shows also the distribution in the six experiments of the average numbers of whorls per plant (3.7 in light and 5.7 in darkness) and of branches per whorl, for all branched plants, (2.1 in light and 2.2 in darkness). The average number of branches per plant for the whole series is 5.1 for the light cultures and 9.9 for the dark cultures (table 1). The average number of branches per whorl for all branched plants is 2.17, which is close to the average for the intermediate whorls (2.19; table 7).

The differences in average number of branches between basal, intermediate, and apical whorls do not hold consistently for each of the six experiments taken separately. Nor do the same relations hold for the cultures in light and darkness taken separately. There is no conspicuous evidence from the plants in these cultures that the sorocarp as a whole tapers either up or down, though there is the slight indication given in the table that the number of branches per whorl tends to increase slightly from base to apex. It is to be remembered that, as noted, the cultures were all grown in the inverted position and that the illumination was not equal on all sides. Experiments controlled for this particular purpose are needed to show definitely whether there is any evidence of taper either up or down.

The data as to the distribution of whorls and branches in the remainder of the branched plants, namely, those with one or two whorls of branches, are given in table 8. The total number of plants in these two groups, taking the fifty light cultures and the forty-nine in darkness is 1106, a little over one-third of the total number of branched plants (3130). There are over twice as many plants with one or two whorls per plant in

TABLE 8

Distribution of branches in plants with one or two whorls per plant

EXP.	PLANTS		TOTALS PER EXPERIMENT				AVERAGES			
			WHORLS		BRANCHES		BR. PER WH.		BR. PER PL.	
	L	D	L	D	L	D	L	D	L	D
I	133	59	195	93	470	219	2.4	2.3	3.5	3.7
II	49	30	70	51	136	122	1.9	2.3	2.7	4.0
III	66	68	110	104	272	260	2.4	2.5	4.1	3.8
IV	91	42	142	63	308	153	2.1	2.4	3.3	3.6
V	255	54	383	78	813	158	2.1	2.0	3.1	2.9
VI	202	57	315	87	653	196	2.0	2.2	3.2	3.4
Totals	796	310	1215	476	2652	1108	2.1	2.3	3.3	3.5
Grand Totals	1106		1691		3760					

the fifty light cultures as there are in the forty-nine cultures in darkness. Experiment III is the only one which shows an excess of plants of this type in the dark cultures and in this case, as the table shows, there were nine light cultures to ten dark cultures. If we allow for this difference the numbers of plants in this experiment also show an excess in the light—sixty-six in light to about sixty-two in darkness. This excess of plants in the light cultures, which is so marked in the group of plants with one and two whorls of branches, gradually decreases (as shown in table 2), till in the group of plants with seven whorls of branches there are more plants in the dark cultures than in the light (69L to 81D). In the case of the numbers of whorls and branches there is a consistent excess in the light cultures over the dark cultures in the case of the groups of plants with one and two whorls of branches each.

The average number of branches per whorl for these plants is 2.1 in the light to 2.3 in the dark (table 8), about the same as for the intermediate whorls in the plants with three or more whorls (2.1 and 2.2; table 7). The average number of branches per plant is 3.3 in light to 3.5 in darkness. These averages of branches per whorl and branches per plant in plants with one or two whorls are both consistent with the evidence that light tends to inhibit the branching of the plants.

The relation of light to number of plants produced is further illustrated in tables 7 and 8. In the columns showing total plants in the light and darkness respectively of the two groups, those with three or more whorls per plant and those with one or two whorls per plant, we find that: in the first group (table 7) 55+ per cent of the plants are in the light cultures to 44+ per cent of the plants in the dark cultures. While in table 8 71+ per cent are shown to be produced in the light to 28 per cent in darkness.

EXTREME DIVERGENCES FROM TYPE

A study of the data indicates that extreme divergences from type can occur in the organization of the sorocarp, and that symmetry in the distribution of the whorls and branches may be present or lacking in varying degrees. I have brought together data as to a number of such extremes in total whorls and branches and number of branches per whorl in table 9. Plants with very large numbers of whorls and branches (table 9A 1, 2, 3) may show great irregularity in the distribution of the numbers of branches per whorl. I have noted the slight tendency to smaller numbers of branches in the basal whorl of sorocarps with three or more whorls of branches. A very considerable number of plants have basal whorls of one branch. In plants A1, 3, and B3, and to a lesser degree in B1 and 2 (table 9) this tend-

ency comes to extreme expression. From four to ten of the lower whorls consist of a single branch each. The average numbers of branches in the basal whorls of plants with three or more branches are 1.7L and 1.6D (table 8).

TABLE 9

Plants showing extreme divergences from type in numbers of whorls and branches per whorl

A. Plants with high numbers of whorls and branches

EXP.	CULT.	
V	607	1. Plant in light with highest no. of branches; has 21 wh. 43 br. *1 1 1 1 1 1 1 1 1 2 1 1 3 2 4 3 4 3 4 3 4 This is also the plant with the highest no. of whorls.
VI	628	2. Plant in dark with highest no. of branches; has 17 wh. 56 br. *1 1 4 5 2 3 4 3 3 4 5 4 4 4 3 3 3
III	460	3. Plant in dark with highest no. of whorls; has 21 wh. 48 br. *1 1 1 1 1 1 1 1 1 1 2 3 3 4 4 4 3 4 4 3 4
		B. Plants with a whorl of six branches
III	465	1. Plant in dark with 15 wh. and 28 br. av. br. per wh. 1.8 *1 1 1 1 2 3 3 2 2 1 1 2 6 1 1
III	466	2. Plant in dark with 10 wh. and 23 br. av. br. per wh. 2.3 *1 1 1 1 1 2 3 6 4 3
III	465	3. Plant in dark with 16 wh. and 30 br. av. br. per wh. 1.8 *1 1 1 1 1 1 1 1 3 3 2 1 3 1 1 3 6
V	617	4. Plant in dark with 10 wh. and 32 br. av. br. per wh. 3.2 *1 2 3 4 2 3 6 4 4 3
III	456	5. Plant in light with 6 wh. and 24 br. av. br. per wh. 4.0 *2 2 4 5 6 5
IV	491	6. Plant in light with 4 wh. and 14 br. av. br. per wh. 3.5 *4 3 6 1

* The order is from base to apex of the plant.

Plants A1 and 3 have apical whorls of four branches, as well as being respectively the plants with the largest total number of whorls in the light and dark cultures. Plant B3 has an apical whorl of six branches. In the other plants of group B (table 9) the whorls with six branches are intermediate. The average apical whorl for all plants with three or more whorls is 2.44L and 2.45D (table 7). The average apical whorl for the four specially selected plants, B2, 3, 4, 5, is 4.25 branches. Plant A2 with 17 whorls and the highest number of branches of any plant observed (56) has its two lowest whorls with one branch each and an apical whorl of three branches.

Its intermediate whorls consist of one with five branches, six with four branches each, five with three branches each, and one with two branches, average 3.4. The average intermediate whorl for all plants with three or more whorls is 2.10L, 2.28D.

I have noted the doubtful nature of the whorls with six branches. The six plants B1, 2, 3, 4, 5, and 6 which showed such whorls range in total number of whorls from 4 to 16. The average number of branches per whorl for the group is about 3 (3.8L and 2.2D). The average for all branched plants is 2.11L, 2.23D (table 1).

Plants with only one branch are found in all of the six experiments (table 6A). There is a total of 110 such plants in the light cultures to 34 in the dark cultures. Such plants come nearest to *Dictyostelium* in their organization and their predominance in the light cultures suggests that the transition from unbranched to branched types is favored by development in darkness.

In comparing such data as the above with similar data for types in which growth and differentiation go on simultaneously it is to be remembered that in these pseudo-plasmodial plants, relative size of the plants, number of whorls, and number of branches in a whorl are determined solely by building, architectonic processes of aggregation, integration, and differentiation and not at all by the regulation of cell growth and cell division. The data indicate that the stimuli which determine the size of the sorocarps operate by regulatory effects determining the area from which the myxamoebae come together to produce a single plant body. These extreme cases of asymmetry in the distribution of whorls, branches, and branches per whorl emphasize the complicated nature of the processes by which the morphogenesis of so simple a plant form as the sorocarp is achieved, but in no way invalidate the evidence that normal alternation of day and night tends to favor the production of more and smaller plants, while relatively continuous darkness tends to the production of larger and fewer plants. This would indicate again, as noted, that cell growth and division go on equally well under both sets of conditions but that the regulation of aggregation and architectonic morphogenesis is such as to tend in darkness to favor the gathering of larger numbers of myxamoebae at each point of integration, thus resulting in the formation of fewer and larger plants per unit culture.

DISCUSSION

Growth. The general results of these experiments indicate a fixity of type in *Polysphondylium* under varying environmental conditions which parallels that in ordinary cormophytic plants. Environment may alter

size, but the type configuration persists. The experiments give further data as to the nature of this type configuration and the range in variation of *Polysphondylium*, and show the general relations of light to its processes of growth, integration, and differentiation.

That free motile cells may, by strictly architectonic or building processes, as contrasted with cell division and cell growth, develop highly specialized and symmetrical organic forms is clear, and that both environmental and hereditary variants are to be found in such types emphasizes the fundamental identity of their morphogenetic processes with those in plants whose cells are in more persistent continuity.

The Acrasieae are very simple plants, and it may perhaps be questioned whether their erect stems and whorls of branches bearing fruiting organs, the sori, justify any comparison with the superficially similar structures of the higher plants. There can be no question, however, that we have here a case of the formation of well differentiated multicellular plant forms in which cell growth and nuclear and cell division are sharply separated in time from the integrating and differentiating processes which result in the formation of the erect radially and metamerically symmetrical plant body. In such a life cycle we can study this integrating and differentiating phase in entire independence of the equally complex processes of cell growth and cell multiplication. This morphogenetic phase involves what is, to use the familiar illustration, essentially an architectural and building process as distinct from the manufacture and accumulation of building materials. It is architectonic in that it is not merely aggregative but involves the production of hereditarily specified plant forms which require for their development characteristic and progressively changing relations of the amoebae to each other and to the external environment quite as in the development of the embryonic cells into the organs and tissues of the mature many-celled plant following the cleavage of the egg. The process is essentially a morphogenesis, but we may characterize it more specifically as an architectonesis, as distinguished from the processes of mass growth and proliferation of cells and undifferentiated cell aggregates, which are also in a sense morphogenetic. It involves both individuating and differentiating processes by which a swarm of isopotential myxamoebae becomes a complex whole comparable to a minute pine tree in its general appearance, and in which the amoebae become ultimately differentiated into organs and tissues, though for a long time maintaining their harmonic equipotential characteristics both singly and as groups.

This organization so achieved is also, as noted above, genetically fixed, and we have a whole series of genera and species in the Acrasieae which seem quite as persistent in their heredity as are plants whose cells are in

more permanent tissue continuity. I have found no reason to doubt that *Dictyostelium* and *Polysphondylium* are genetically distinct, though the unbranched forms of *Polysphondylium* are so similar to *Dictyostelium* in their general plan and appearance.

That the architectonic phase in development is distinct and specific is very clearly shown in the results of so-called tissue culturing and transplantation as developed by the Carel and Spemann schools. Just why the cells of a bit of chick embryo should maintain cell growth and cell proliferation indefinitely and show little or no tendency to form tissues, or organs, or an entire chick, while a bit of hydra so readily undergoes morphallactic individuation, and the cells of a bit of begonia leaf not only grow and divide but integrate and differentiate into a complete begonia is, of course, as yet not all clear. The much more successful, though frequently bizarre, development of tissue or organ transplants indicates, however, that the processes involved are all in their nature, in part at least, intercellular space and chemical hormonic relations. I have discussed these relations more fully in connection with the reactions involved in stipe formation (1929, 1926). But however such integrations and differentations are to be explained, the results so far obtained with tissue cultures make very plain the distinction between cell growth and proliferation and the integrative and differentiating processes by which a mass of cells becomes a unit organism with differentiated organs and tissues, and it is this distinction which is so clean cut in the life cycle of these semicoenobes.

In *Polysphondylium* the stages of growth on the one hand, and of integration and differentiation on the other, almost, as it were, constitute an alternation of generations, so sharply are they separated both as to time and the nature of the processes involved. Still the basic process in building the sorocarp is amoeboid cell motion quite like that occurring while growth and division are going on, but highly determinate and specified cell motion as contrasted with the wanderings of the myxamoebae at the earlier period, though perhaps no less environmentally controlled if we take into account the intercellular environment.

Tropisms. I have referred the direction of anallactic development to such stimuli, as light, gravitation, moisture, etc. Olive (1902) and Potts (1902) have noted a tendency of the pseudoplasmodium to grow away from the substratum under conditions where the orienting factor or factors are not clear. In certain cases I have noted such phenomena under conditions which also certainly need further study. I have frequently observed plants in an inverted Petri dish dung agar culture which had built down vertically forming branches as usual till they reached the glass cover

of the dish, and then have turned and built almost vertically back upwards from the glass (exotropy). The glass seemed quite dry. There was no evidence of negative hygrotropism. The reversed stipe shows no branches till it has reached something like the length of the basal segment generally. Though similar, such cases are not to be confused with those in which a plant lops over until the developing sorogen touches the nutrient substratum and then changes its direction of anallactic advance until it regains a position more nearly vertical to the substratum. This may be an ordinary hygro-geotropic response. In the special cases here referred to the sorogenic mass reverses its direction of motion on contact with a glass surface toward which it had just before been advancing.

Types. On the assumption that *Polysphondylium* tends to produce a certain type of plant as to number of whorls, distance between whorls, angle of deviation of branches, number of branches per whorl, etc., the total number of branches, for example, for each group of plants with increasing numbers of whorls (table 4) should increase till the type number is reached. This is true as shown by the data given in the table, and it would appear that the type plant has about three or four whorls of branches with between two and three branches per whorl. Above this type the total number of whorls or branches per group should and does decrease, with a decrease in the number of plants.

The data indicate that the stimuli which determine size and form must operate as noted by regulatory effects, either determining the extent of the area from which the amoebae come together to produce a single sorocarp or their abundance in any given area of the nutrient substratum. The fact that normal alternation of day and night tends to favor the production of more and smaller plants while relatively continuous darkness tends to the production of larger and fewer plants, may be taken to indicate that cell growth and division go on equally well under both sets of conditions but that the processes of aggregation and integration tend probably in darkness to favor the gathering of the myxamoebae from larger areas to the points of integration, thus resulting in the formation of fewer and larger plants per unit culture.

Photomorphosis. As noted, Potts (1902) has observed that light tends to reduce the size of the plants of *Polysphondylium*, and he gives the height of the tallest he obtained in light as 6 mm., with three or four branches, and in darkness as 13 mm., with twelve branches. He thus leaves the problem of light effects on growth without discussing the question as to whether it is a matter of relative cell proliferation or cell aggregation or both. Potts also gives a mass of data as to the symbiotic, temperature, moisture, and other relations of these plants. All of these relations, however, have to do

more especially with nutrition and growth, rather than the morphogenetic processes with which my studies are especially concerned, and I shall hence not discuss them further in this connection. Of particular interest are his observations on the effect of an optimum rate of transpiration on the height of the plants.

Stameroff (1897) finds that the vegetative hyphae of *Mucor* and *Saprolegnia* grow equally well in light and darkness, while light tends to check the growth of the fertile hyphae of *Mucor*. It will be interesting to know whether the upward so-called growth of the fertile branches of *Mucor* involves nuclear division and increase in dry weight, or merely the well known streaming of protoplasm from the vegetative hyphae into the reproductive branches. In the latter case the differential effect of light on growth vs. building processes would be the same for *Mucor* as I have found it for *Polysphondylium*.

The observed numerical data for the 50 cultures grown under conditions of alternating day and night, and the 49 cultures grown in relative darkness, are summarized in table 1, and the results obtained, when allowance is made, for the extra culture in light, are given on page 16. The evidence shows that, as Potts discovered, the plants grown in darkness are larger. However, it is also shown that the number of plants is greater in the cultures grown in alternating day and night, so that the total numbers of whorls, branches, and sori produced are about the same. There is a difference of only from one to two per cent (p. 16), while in the number of plants there is a difference of about 29 per cent in favor of the cultures grown under conditions of alternating day and night. The cultures in the light produced 64 per cent of the total number of individual plants, against 35 per cent produced by the cultures in the dark. But the 35 per cent of the plants in the dark produced practically the same percentage of the total number of whorls (50%L, 49%D), branches (48%L, 51%D) and sori (50%L, 49%D) as the 65 per cent of the plants in the light (p. 63).

The percentage of terminal sori, as compared with lateral sori, is greater in the light than in darkness by about 7 per cent owing to the relatively larger number of unbranched plants and those with few branches. The terminal sori of branched plants are larger in general than the lateral sori. I have no data as to the relative diameters of the terminal sori of unbranched plants. Whether the ratio of spores to stipe and branch cells (germ cells to somatic cells) is greater in the unbranched or the branched plants—in the plants grown in light or in darkness—must be determined by further quantitative studies.

The difference between the number of unbranched plants (*Dictyostelium* type) in the light (1025, 74%) as compared with those in the dark

(354, 25%) is (671, 48%). That is, there is an excess of unbranched plants in light of 48 per cent. The difference in the number of branched plants in the light (1915, 61%) and the branched plants in darkness (1215, 38%) is 700 (22 per cent). That is, there is an excess of only 22 per cent of branched plants in the light. It would appear that under the conditions of my experiments, the production of wholly unbranched *Polysphondylium* plants (*Dictyostelium* type) is favored more than twice as much in the light as is the formation of branched plants. This does not, of course, prove that all unbranched plants could be made to disappear by growing the cultures in absolute darkness.

The fact that fungi respond to environmental stimuli of light, heat, chemical conditions of nutrition and stimulation, etc., in highly specific fashions both as to form, rate of development, kind and time of fructification, etc., has long been recognized, and latterly the effects of nutritive and chemical stimuli in producing form changes, so called mutations, has become a subject of special interest. That light particularly has specific and very various effects on the development of the fungi, though unlike green plants they are quite incapable of using it directly as a source of energy in their metabolism, was early noted. The earlier data have been well summarized and analyzed by Elfving (1890), who interested himself especially with the comparison of these light effects in fungi with the phenomena of etiolation in green plants. Zopf in Schenk's *Handbuch* (1890) also brings together the results of Wettstein (1885, p. 39), Klein (1885), Brefeld (1889), Winter (1874), Kraus (1869), and others on the light relations of the fungi. The photomorphotic relations of the higher fungi particularly are extremely varied and frequently bizarre to a degree hardly equalled in the etiolated forms of green plants. Just why a certain species, *Pilobolus oedipus*, can go through its whole life cycle quite normally in darkness, while another, *P. microsporus*, is entirely dependent on light at certain stages (Brefeld, 1889), is as yet quite as little understood as is the whole matter of the effect of light on growth rates in green plants. As, however, the observations of the authors cited for the most part do not extend to an attempt at analysis in terms of cell behavior, I shall not refer to them further at this time than to point out the attractive nature of this field for further study.

Heredity and morphogenesis. The intensive study of the germ plasm and the results achieved by genetical analysis have led naturally, as pointed out by Sinnott, to the effort to trace the direct relations of the germ plasm to the development of the characters of the adult plant. Sinnott and Durham (1929) find that fruits of cucurbits of various shapes and sizes all have cells of the same shape and size. This confirms the work of

Amelung (1893) on the relation of cell size to organ size, and that of Tenopyr (1918) on cell form as related to organ form.

However, in this very careful and critical study of the development of form the authors note that, while the shape of the cells tends to be essentially the same (p. 418) in mature elongate, rounded, and disk shaped fruits, in certain tissues—'inner tissue continuous with the carpellary folds' and 'outer layer'—the cells are respectively 'elongated longitudinally and elongated tangentially' in a fashion which may well indicate directions of cellular mass growth. This suggests that while the form of the mature cell is so to speak standardized independently of fruit form, the growth axes of elongation by which the specific form of the fruit is achieved may be indicated by the form of the cells. It also suggests further that the orientation of the division planes (census growth) may follow rather than precede the determination of the axes of mass growth of the cells.

It is such facts as these which might lead to the conception that mass growth is quite independent of cell division, which may then be assumed to follow in accord with the simple Sachsian principles of rectangular intersection, least surfaces, etc. However, the cell can divide successfully whether it is isodiametric or elongated. It can grow for a time at least equally well either in three dimensions or in one dimension. The cell may show specific inherited characteristics, both as to plane of division and axes of growth enlargement. These relations are amply illustrated in the growth and multiplication stages of the myxamoebae and other types of amoebae, swarm spores, and protophytes generally. The plane of cell division may be controlled by external environmental factors of light, gravity, etc. The direction of movement of free cells or the direction of elongation of attached swarm spores may also be very easily controlled by external environmental stimuli of light, heat, chemical agents, etc.

When such free cells are aggregated in the tissues and organs of many celled plants or animals the new factors which appear are intercellular relations of contact, pressure, chemical stimuli, etc., and it is natural to conclude that these new internal environmental factors, with those of the external environment, have much to do in determining the form and internal functional interrelations of the cells of the multicellular organism, that is, with both individuation and differentiation. That any further principle of organic protoplasmic unity appears in multicellular organisms as such, remains to be proven.

In the architectonic processes of coenobes and semi-coenobes the axial differentiation of the stipe and branches are seen directly to be matters not of protoplasmic mass growth but of specifically oriented movements of independent cell units, the resulting form of the whole being deter-

mined by their coordinated and integrated behavior. Sinnott's (1929) careful observations show clearly that at the critical steps in fruit morphogenesis the specificity of cell form comes to expression even though the specific shape of the mature fruit is in no particular determined by the shape of its cells. Speaking of the development of the ovary wall he says:

> Certainly in many cases a rather definite line is visible in early stages of the ovary, between an inner tissue, clearly continuous with the carpellary folds and consisting chiefly of cells *elongated longitudinally*, and an outer layer in which most of the cells are *elongated in a tangential direction*.

These are apparently growth directions indicated directly in the form of the cells and achieved by them in their mutual interrelations. The fruit, as Sinnott shows, is from a very early stage a tissue aggregate consisting of (1) epidermis, (2) cortex, (3) vascular ring, (4) medulla, (5) sterile carpellary tissue, and (6) ovuliferous carpellary tissue. Each such tissue consists of cells of more or less specific shapes which suggest axes of differential elongation analogous to those in amoeboid form changes of specific types.

It is obviously the cellular organization which makes possible all this tissue differentiation. The problems of morphogenesis include all of these interior as well as the more external form specificities and their existence marks very sharply the advance from the architectonic possibilities of the true plasmodium and coenocyte to the complexities of histogenesis and organogeny in the higher plants.

The existence of such architectonic processes as are found in the semicoenobes *Dictyostelium* and *Polysphondylium* with their pseudoplasmodia of free independent amoeboid cells certainly suggests that the evolution of morphogenetic processes consists in the development of progressively more specific aggregations and integrations of cells rather than in the growth and differentiation of a plasmodial protoplasmic mass whose form, aside from postulated internal form determining tendencies, must be achieved by the surface relations of a single continuous plasma membrane. Sinnott (1922, 1927) emphasizes that the factors which control morphogenesis in cucurbits are not mass or dimensional factors but are essentially factors determining the form, i.e., the *distribution* in space of the unit products of cell growth and multiplication. The differences between a Fordhook and a disk fruit are essentially matters of the distribution of a given number of cells of essentially equivalent form and size. The problems of morphogenesis become thus matters of cell movement and distribution quite as they are in *Polysphondylium*.

Growth hormones. The relations of light to growth have been the subject of very intensive experimentation in recent years. Following the determination of the localization of receptive and motor regions has come

the discovery of the nature and the means of transmission of stimuli from the specially sensitive apex to the elongating motor regions. Went's (1927) demonstration of so-called growth stuff as the product of stimulation and the agency of its transmission has brought the phenomena of phototropic bendings into line with the hormone theory of secretory and growth control in animals. Went's use of the term growth stuff rather then hormone emphasizes the closeness with which the problems of growth and tropistic bending have been interrelated in the minds of students of the light relations of plants. As I have pointed out (1929) *Polysphondylium* shows characteristic and typical tropistic curvatures without either mass growth or cell multiplication being involved in any way. The phenomena are directly tropomorphotic rather than, as generally described, growth curvatures, the stimuli being apparently those of negative hygro-geo- and perhaps exo-tropy. The familiar cases of so-called tropistic growth curvatures in root and shoot apices may be interpreted as matters of oriented amoeboid elongation of the cells in the stretching region back of the apex. This tissue elongation it is agreed is a matter of vacuolization of the cells without necessary increase in dry weight, and it is of great interest that Olive (1902) emphasizes the enlargement by vacuolization of the myxamoebae as a factor in the building of the stipe tissue. In the upturning sorogen of *Polysphondylium* after contact with the substratum the curvature is as noted (1929) strictly due to the tropistic change in direction of movement of each one of the mass of creeping myxamoebae.

Noll, following Godlewski (1879) and others, claimed to demonstrate experimentally that such bending is due to the greater elongation of the cells on their convex flanks resulting from the increased elasticity of the cell walls on that flank. Went (1928) accepts this earlier theory of the unequally changed elasticity of the cell flanks as a mechanical basis of the bending process. Others have claimed that turgor differences between the cells on the two flanks of the bending shoot are involved. However that may be, it seems to me plain that in both *Polysphondylium* and the higher plants the curvature is not a growth process either in the sense of cell multiplication, which I have called census growth, nor in the sense of increase in volume or dry weight of the protoplasmic mass involved. What is common to both cases is specifically oriented cellular protoplasmic motion. In *Polysphondylium*, as noted, a tropistic change in the relative rate of creeping movement of the individual amoebae on the two flanks of the sorogen is the means by which the upward curvature is achieved.

The evidence that certain regions or tissues in the embryo serve as centres for the control of morphogenetic processes as developed by Spemann (1923) and others seems well established. That the root tip and

other embryonic regions in the higher plants function as special receptors was pointed out by Charles Darwin (1880) and has been many times confirmed. Went (1927), as noted, has recently obtained evidence that this control in the coleoptile of the oat is effected by the formation and migration to motor regions of what he calls growth stuff. Pincussen (1930), in his valuable and interesting analysis and summary of the light relations of organisms, concludes that:

Bei solchen Reaktionen, welche den Gesamtorganismus beeinflussen, wird in den weitaus meisten Fällen die Bildung eines chemischen Körpers angenommen werden müssen, der in einer Anfangsreaktion entstehend nun an der betreffenden Stelle seine Wirksamkeit entfaltet. Alles, was nach der Bildung dieser photochemisch erzeugten Substanz geschieht, würde sich ebenso abwickeln, wenn ein solcher Stoff auf anderem Wege entstanden wäre. Anfänge, solche photochemisch entstandenen Körper zu identifizieren, sind in sehr bescheidenem Masse vorhanden. Es ist das Gebot der Stunde, solche Stoffe zu isolieren und dadurch die Wirksamkeit des Lichtes zu beweisen.

In these semicoenobes functional and tissue differentiation apparently have not proceeded so far as to involve the development of such centres of control. That myxamoebae influence each others movements and differentiations by chemical substances which they produce and set free seems, as I have pointed out, to be a natural assumption, especially for the period of their synallactic assembling. The chemical as well as the contact and pressure relations of the cells in the pseudoplasmodium at all stages of its formation and development into the sorocarp call for careful study. We have in *Polysphondylium* the appearance at least, of a cormophytic system which is influenced photomorphotically in its entirety (size and proportions). And the possibility is suggested that apparently systemic effects may be produced through intercellular reactions which are local, both in their initiation and their final expression. It would seem that *Polysphondylium* is an organism in which metameric differentiation, the formation of branches in whorls, the characteristic rate of taper in stipes and branches, may all be achieved locally by cellular interactions controlling cell movement and differentiation.

Etiolation. It was early recognized that the basic problem in etiolation is whether its common expression, in excess increase in length of the internodes and relatively small size of the leaves, involves, for the stems, increase in the number of cells by division (census growth) or increased mass growth and length of the individual cells, and for the leaves whether their relatively small size is due (1) to their having fewer cells or (2) smaller cells or (3) whether both differences exist in leaves of etiolated plants as compared with leaves on plants grown under normal conditions of illumination. It can hardly be claimed that the study of either of these problems has led

as yet to final and convincing results. MacDougal (1903) has summarized the data from the literature very adequately.

It is of interest to note that in the attempt to analyze the effect of light in checking growth and inducing bending, Vines (1886, p. 398) on the basis of his earlier work in Sachs' laboratory, develops the conception that:

> The effect of the tonic action of light in diminishing the rate of growth is probably to be ascribed to an interference with the motility of the protoplasm of the growing cells. We may regard then, the tonic action of light, manifested in its retarding effect upon the rate of growth as an inhibitory action, and as being due to the induction of a certain degree of rigidity in the protoplasm, the rigidity being slight at low intensity and gradually increasing with the intensity until under the influence of very intense light, it is complete.

Again (p. 581) in discussing the curvature of unicellular organs Vines says:

> Without pretending to say precisely what these changes may be, we may suggest that they consist in a modification of the motility of the protoplasm on one or both sides of the cell. The motility of the protoplasm of the concave side may be diminished or that of the convex side may be increased, or as is more probable, in view of the curvature of multicellular organs both of these effects may be simultaneously produced.

The possibility that these conceptions of Vines are in line with the evidence given by Lepeschkin (1931) and others that light increases the permeability of protoplasm is also to be considered.

Baranetzky (1875) reported that plasmodia tend to become immobile and ultimately abnormal in light, forming more thickened masses or strands as compared with the delicately reticulated appearance of normal plasmodia.

Brotherton and Bartlett (1918) studied the length of the epidermal cells of normal and etiolated epicotyls of *Phaseolus multiflorus* and conclude that the specific mean length of cells about to divide is the same in both light and darkness. However, as a result of excess increase in length of etiolated cells 34 per cent of the increase in length of the etiolated epicotyl is to be accounted for by increased cell division. It would seem that, since cell division only affects length where followed by cell elongation, this 34 per cent might best be called secondary etiolation resulting from a law of specific cell size. The basic factor in etiolation (66%) would then be, according to their argument, increased elongation of cells in darkness. They find that darkness favors, and light retards, elongation and has no direct effect on the rate of cell division. This is true of *Polysphondylium* if cell elongation and cell movement can be found to have a common basis. However, Brotherton and Bartlett find also that certain elongated meristem cells grown in darkness and not showing 'secondary divisions' may have a mean length of 0.149 mm., not well below but above the length of undivided cells (0.140 mm.) grown under ordinary conditions of illumination, which they explain as due to slenderness or some other cause.

Organization. When, as in the higher plants, the cell units remain in tissue continuity as they grow and divide it may seem possible to regard the whole cormophyte from its beginning in the egg as an integrated and differentiated mass of protoplasm. That this is not true of either the swarm of myxamoebae or the pseudoplasmodia and that the individual amoebae are in no sense specified as to their position in the finished sorocarp is obvious and can be illustrated experimentally by showing that out of a given swarm without further growth or cell divisions either one or several sorocarps of varying sizes and proportions may be made to develop. The process of aggregation and integration may be interrupted after it has been initiated and is well advanced and the number of ultimate unit systems may be changed.

It is obvious that we are here observing the actual process of achieving a higher unity by the free reactions and interactions of a swarm of units of a lower order whose individual unity and organization in no way resemble or directly represent the type of unity achieved in the sorocarp. The myxamoeba is a uninucleated mass of protoplasm creeping by pseudopodia and dividing more or less periodically. The sorocarp is an erect, radially and metamerically symmetrical structure simulating the cormophytic organization of the higher plants. The passing from one stage to the other is visible integration, the expression of the inherited and spontaneous activities of the amoebae limited and more or less directed by internal and external environmental stimuli.

It gives me pleasure here to express my high appreciation of the very faithful and efficient work of my research assistant, Dr. Florence E. Meier, who made the cultures and did the laborious work of recording and tabulating the results. I am also much indebted to my wife, Helen S. Harper, for assistance in checking and tabulating the results. Tables 6A and 6B were drafted by her from the original protocols.

COLUMBIA UNIVERSITY
NEW YORK

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The genetics of Neurospora

I. The inheritance of response to heat-treatment

CARL C. LINDEGREN

(WITH FOUR TEXT FIGURES)

In the Ascomycetes, the eight ascospores, usually produced in a durable sac, are the result of meiosis of a single nucleus. Moreover, the sacs in some species are long and narrow. The products of division are not jumbled together, but are arranged in a row, so that their positions indicate their relationships. Micromanipulation and aseptic culture technique enable one to isolate these ascospores in order, and to study the genetic characters of the mycelia resulting from their germination. But the question of nuclear fusions and reductional divisions in the Ascomycetes has received many contradictory interpretations at the hands of different writers. The same form has been described as being parthenogenetic, or as having a single sexual fusion, or a double sexual fusion. Scarcely less divergent are the interpretations of the divisions in the ascus. There seems to be a general agreement that the chromosomes undergo what resembles a meiotic division, but the smallness of the chromosomes, and the difficulty of counting them, has led to a variety of interpretations.

An analytical knowledge of the genetics of an organism depends upon coördinating the nuclear history with the genetic behavior. This means that before the mechanism of meiosis can be studied by means of the arrangement of the ascospores in a long ascus, nuclear history and sex phenomena must be known accurately. Once they are understood, the Ascomycetes should provide excellent objects for studying the immediate effects of meiosis.

MICRODISSECTION AND CULTURE METHODS

Dodge (1928) and Wilcox (1928) began the genetic study of the ascomycetous genus *Neurospora* by isolating the spores of individual asci. They germinated each ascospore separately and cultured its mycelium alone. In order to continue this work, their methods have been refined. Microdissection by hand has been abandoned and a micromanipulator (text fig. 1), which is more readily adjustable than screw micromanipulators, has been devised. It is also much cheaper, being made chiefly of wood and containing no screws. Its base is a wooden block 50×15×5 cm. In the midline of the block there is a groove 2 cm. deep, 2 cm. wide, and 10 cm. long. A steel rod 60 cm. long, 1 cm. in diameter, fits solidly in a hole bored vertically through the groove at the side which will be nearest the microscope. A brass tube, 1 cm. inside diameter, slides easily up and down this

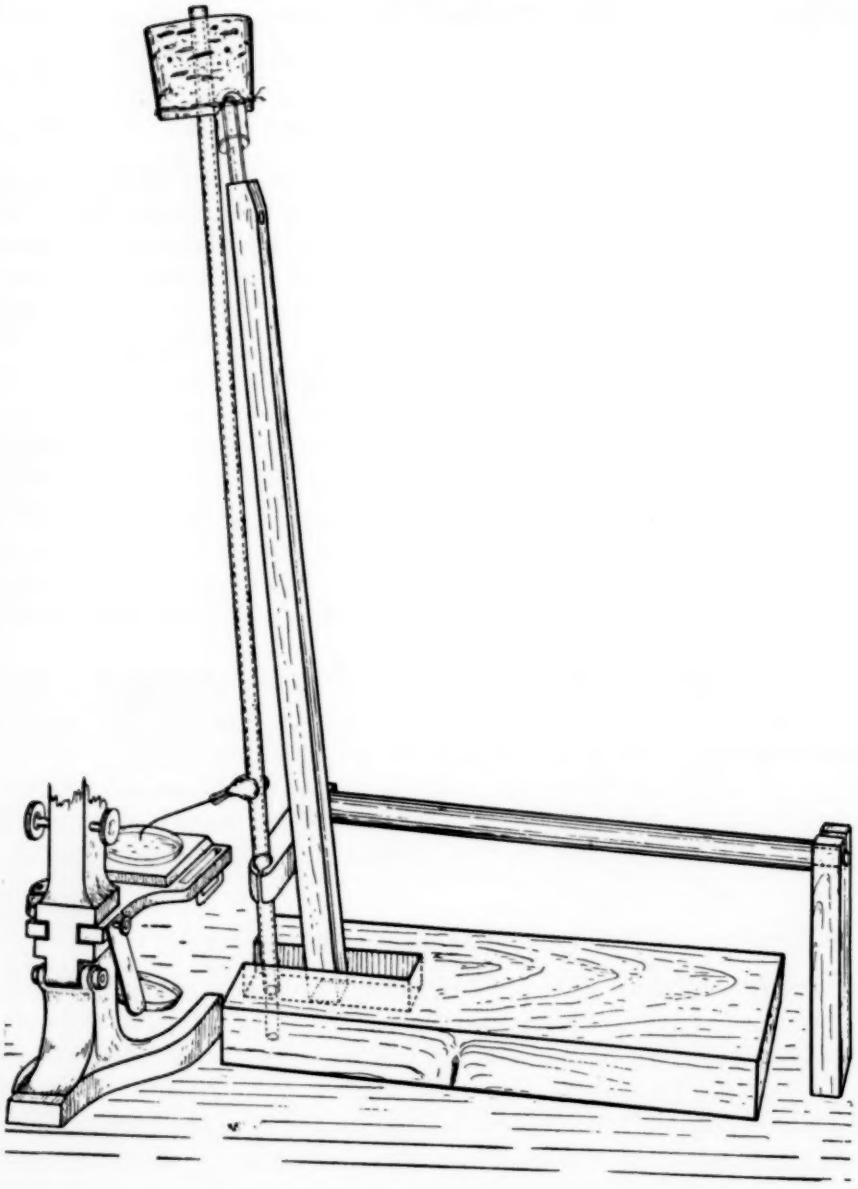

Fig. 1. Wooden micromanipulator ready for use. The needle is set by hand so that it just clears the agar when the slanting rod is close to the brass tube. Then by moving the rod away from the tube with the right hand and pulling the plate away with the left hand, a single spore may be 'hoed' out of the ascus. This instrument with some improvements can be obtained from Mr. L. L. Konrad, care of Spencer Lens Co., San Francisco, California.

rod. Three-eighths inch curtain rod and three-eighths inch inside diameter brass tubing, obtainable in any hardware store, are sufficiently accurate. A needle, inserted in a short glass rod, is stuck to the brass tube with modeling clay so that it just clears the surface of the microscope stage. Movement of the needle in an arc about the vertical steel rod is obtained by a wooden lever pointing away from the operator. The base of the brass tube fits solidly in this wooden lever. The direction of motion of this lever is changed by attaching at right angles a one-half centimeter wooden rod placed so that its free end is conveniently situated at the right hand of the operator. The free end of this rod is fitted solidly in an upright wooden handle which rests upon the table. The wooden rod must have enough spring so that it will allow the handle to rest on the table while the brass rod is being moved upward and downward. Sensitivity of the movement in the arc about the steel rod depends upon the length of the needles relative to the length of the wooden lever, by the principle of similar triangles. For moderate magnifications one to five sensitivity is sufficient, using a 10 cm. needle and a 50 cm. lever.

Movement of the needle point in a vertical direction is obtained by the following device: A large cork is firmly fitted on the upper end of the brass tube. A 2 cm. test tube, which has been cut off about 2 cm. from the base, is fitted solidly in a hole in the base of the cork. A wooden bar $50 \times 2 \times 2$ cm. stands obliquely, and its lower end slides in the grooved base. Its upper end is tapered and rounded to articulate in the cup formed by the base of the test tube. Up and down movement of the needle is obtained by sliding the lower end of this bar along the groove, toward or away from the vertical rod. To determine the sensitivity of this movement, let the length of the oblique bar which forms the constant hypotenuse of a variable right triangle be equal to a. Let the horizontal side (groove) equal x and the vertical side (brass tube and a steel rod) equal y.

$$\text{Then } x^2 + y^2 = a^2$$

By implicit differentiation with respect to x

$$\frac{dy}{dx} = \frac{-x}{y}$$

The sensitivity thus depends inversely upon the ratio of the variable horizontal side to the variable vertical side of the triangle. Therefore, the horizontal side must be short to give maximum sensitivity. It is possible to work with a horizontal side of about 1 cm. with the vertical side of about 50 cm., thus minimizing the manual movement about fifty times.

Chromel needles were used. They were made by hammering a piece of wire very thin and cutting to a fine point with scissors. After the last cut

the wire was again hammered to produce a hoe-like end rather than a pointed one.

The dissections were performed on clear three per cent agar, as follows: Perithecia were crushed between flamed microscope slides and a drop of water was added from a sterile lip pipette. The asci emerge from the perithecia in groups and the clusters can be easily picked up with the pipette and placed on the agar. These asci, still attached to the ascogenous hyphae, spread out radially on the agar. Then individual asci are dissected off as soon as the drop has dried out. The location of the spores in an ascus is indicated by numbering from one to eight; the spore at the outer end of the ascus being number one. The thin but broad hoe-like end of the needle is dropped between the first and second spore and the plate pulled away by hand, thus breaking the ascus wall. A mechanical stage is not necessary to hold and move the plate, although some workers might desire it. All eight spores are dissected—'hoed' out—one by one, and placed in a row. Then they are transferred individually to a second plate set up under a second binocular, so that there is no difficulty in returning to the row of ascospores in the first plate. The position which a spore occupied in the ascus is indicated by a number marked in the agar beside it.

Heat-treatment is used to germinate the spores. They are then transferred individually to agar in small tubes (9 by 100 mm. or 3/8 by 4 inches) by cutting out the small piece of agar containing each germinated spore under a binocular microscope. The heat-treatment kills any conidia which might have been transferred with the ascospores into the agar plate. The transfer of the germinated spores to small tubes is made in a $3\times3\times6$ foot glass chamber which has been liberally sprayed with dilute alcohol and wiped down with mercuric bichloride solution.

The small tubes are kept in racks made of pressed cork strips. This pressed cork is 3 mm. thick and is cut in strips 2×30 cm. About sixteen holes, each 9 mm. in diameter, are punched in the strips with a cork borer. The culture tubes are inserted in the same order in which they occur in the ascus and are held tightly enough in place so that they cannot fall out. These strips of tubes are mounted in wooden racks.

HEAT-TREATMENT METHODS

Dodge (1912) has shown that the ascospores of certain species of the Ascobolaceae, which do not ordinarily germinate in the laboratory, can be induced to germinate by heating. The spores are placed on agar in Petri plates, and the plates are put in a gas oven, which is then regulated, so that the temperature rises to about 70° C. in about twenty to thirty minutes. The plates are then removed. In some species, some spores fail to

germinate even after this treatment. Dodge has since shown, in other papers, that this method may be employed successfully in inducing ascospores of certain species of *Aleuria*, *Lachnea*, and *Neurospora* to germinate. This method has also been employed by Ramlow (1915), Betts (1926), Wilcox (1928), and others. The writer has conducted a series of experiments on ascospore germination and describes below a more refined, but not necessarily infallible, method of heat-treating spores of *Neurospora*.

Experiments to determine an optimum heat-treatment

In studying heat-treatment, spores of *Neurospora tetrasperma* were placed on agar in small Petri dishes. The depth of the agar varied from three to seven millimeters. The dishes were placed on a one-eighth inch thick copper plate, set on top of a Columbia paraffin oven. The copper was allowed to come to a stable temperature (73–77° C.) before the Petri dishes were placed on it. A few drops of water under each dish insured a good contact. The temperature at the surface of the agar gradually rose from the original room temperature of about 20° C. to nearly 70° C. Temperatures were determined by the melting points of various organic compounds. These were placed on thin cover glasses, a series of which were laid on agar in Petri plates containing no spores. After many trials, the compounds shown in table 1 were selected. The compounds began to melt

TABLE 1

Compounds used as thermometers

COMPOUND	MELTING POINT	TIME OF MELTING IN MINUTES	
		Plate 1	*Plate 2*
Benzophenone	47.5°–48.5°C.	6.5– 9.0	8.0– 11.0
Betabromnaphthalene	53.0°–55.0°C.	32.0– 34.0	38.0– 42.5
Betachlornaphthalene	56.5°–57.0°C.	39.0– 40.0	49.0– 51.0
Palmitic acid	61.0°–61.5°C.	61.0–100.	75 –105
Stearic acid	69.0°–69.5°C.	240	240

at the lower temperature given and were completely melted at the higher temperature. The table also shows the times at which the compounds melted in two blank plates, of different thickness of agar. Thus the approximate temperatures of the ascospores on the surface of the agar on another plate, heated simultaneously with the 'thermometer' plate, were determined. The main errors of this method are: (1) raising the cover of the 'thermometer' plate to wipe off the moisture film, for observation of melting points, obviously cools this plate below the temperature of the spore-bearing plate; (2) the lowering of the melting point of the organic compounds due to mixture through distillation; (3) the irregular heating

of different plates due to differences in size, agar depth, position, contact, etc. It is questionable if a better method for determining the temperature would be worth the trouble, because the variation among the several plates is probably considerable. A series of experiments was performed to determine the optimum time of heat-treatment. Sixty-seven Petri dishes containing spores of *N. tetrasperma* were treated for different lengths of time on the copper plate. Blanks were used with the organic compounds as thermometers. The results corresponded roughly with those shown in table 1. After sufficient time was allowed for germination of all spores capable of germination, counts were made. Each percentage recorded is the final count from a single plate containing from forty to several hundred spores, generally more than a hundred. Table 2 shows the data, and text figure 2 is a graph showing the relation between the duration of heat-treatment by the copper plate method and the percentage of germination. These are the total data from sixty-seven Petri dishes, each containing on the average well over a hundred ascospores.

TABLE 2

Germination counts on sixty-seven Petri dishes containing Neurospora tetrasperma ascospores heat-treated by the copper plate method for various lengths of time

TIME MIN.	PER CENT	TIME MIN.	PER CENT	TIME MIN.	PER CENT	TIME MIN.	PER CENT
11	2	25	100	46	98	90	95
12	0	26	92	48	99	100	97
13	0	26	97	50	97	110	92
14	7	27	100	52	85	120	93
15	1	28	96	54	93	130	82
16	3	29	95	54	98	140	94
17	0	30	98	56	56	150	78
18	2	30	100	58	78	160	0
18	4	31	97	59	94	170	35
19	2	32	98	62	88	180	11
19	30	33	93	66	81	190	8
20	81	34	98	68	92	200	0
21	88	34	95	70	97	210	1
22	63	38	99	74	93	220	1
23	77	40	98	78	97	230	2
24	84	42	80	82	93	240	3
25	86	44	98	86	97		

In analyzing text figure 2, it can be seen that the optimum heat-treatment has a rather long range, namely, from twenty-five to forty minutes. It may be concluded that a heat-treatment of from twenty-five to forty minutes, with the temperature rising from approximately 54° to 58° C., will give very high percentages of germination. There is evidence to show

that lower temperatures for longer times will give the same results, but no effort has been made to determine a critical temperature.

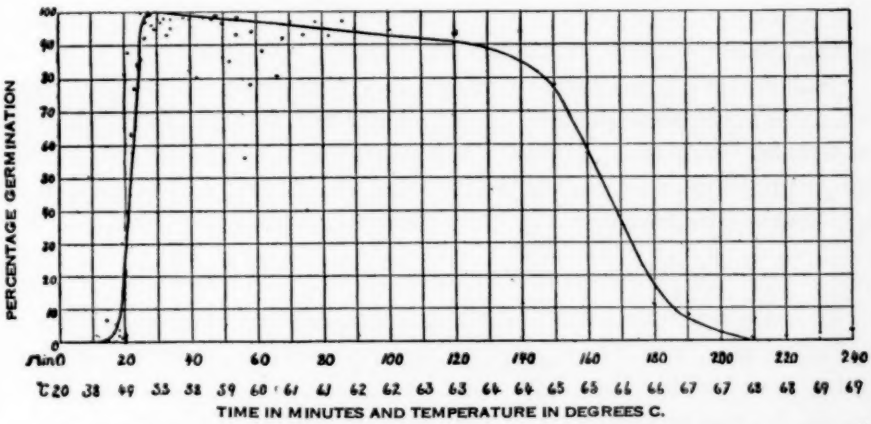

Fig. 2. Graph showing the percentages of germination obtained in 67 different agar plates placed on the copper plate for various lengths of time. The temperature of the agar during the time the dish was on the copper plate is also shown. Note the flat maximum and the rapid increase beginning at about 20 minutes, as well as the rapid fall after 140 minutes.

Experiments on killing conidia

The peculiar advantage of using *Neurospora* for genetic studies lies in the fact that the conidia and mycelia, which may be accidently transferred with the ascospore, are killed by the heat-treatment which induces the ascospore to germinate. After the ascospores have germinated, fifteen to eighteen minute heat-treatments by the copper plate method are sufficient to kill the mycelium of the germinated spores.

In these experiments an eighteen minute treatment raised the temperature of the growing mycelium from room temperature to about 48° C. Later a thermostat-controlled chamber was used instead of the copper plate for heat-treating the spores. The temperature was constant at 58° C., and the plates were treated for sixty minutes. To test whether this treatment was sufficient to destroy the conidia, young conidia of *Neurospora tetrasperma* were treated. The untreated conidia showed 100 per cent germination on cornmeal agar. Ten minutes in the thermostat at 58° C. delayed germination for as long as two days, but practically all of the conidia eventually germinated. From 45 to 70 minutes heating at 58° C. killed all the conidia in thirteen of the small plates used, and no later growth was produced, although they were held for ten days. In two cases out of fifteen, however, (one Petri plate held for sixty minutes and the other for sixty-

five minutes) the plates were overgrown when examined five days later. It is probable that only a small number of conidia survived in these plates. In general the asci are carefully washed and no conidia are carried over. The chances of any conidium, which might accidently be carried over, surviving the sixty minute treatment are evidently not great.

Random single ascospore cultures of *N. crassa* were often made by sowing conidia and ascospores on an agar plate and allowing the conidia to germinate. Then the plate was heat-treated and the ascospores transplanted to tubes. As a control many of the germinated, and subsequently heat-treated, conidia were also transplanted, but none ever grew.

INHERITANCE OF 'RESPONSE TO HEAT-TREATMENT'

In the present method of heat-treating ascospores, the copper plate is no longer used. It was thought that longer treatments, at lower temperatures, gave greater reliability with less injury, and hence fewer killed spores. A small thermostat was built for this low temperature treatment. In the early part of the work, it was thought that cooling the Petri plates containing the spores down to about 10° C., for 12 to 24 hours before heating, increased the certainty of germination. Recent experiments show that cooling has no such beneficial effect.

It was determined that the optimum heat-treatment for a cooled Petri plate in the thermostat held at 58°C. was one hour. The thermostat is so small that its temperature drops to about 45°C. on introducing the cooled plates, and does not attain 58°C. until about 20 minutes later. It may be that the ascospores on the surface of the agar never quite reach the temperature which the thermometer in the thermostat indicates.

In the course of the work the spores from over 500 individual asci were isolated in order, one by one, so that their positions in the particular asci were known. Only asci containing eight ripe spores were used. These spores were incubated on agar over night at room temperature to determine if any germinated without heating, and if any conidia had been carried over with them. At first all plates were cooled as above noted before heat-treating, but later the cooling was abandoned. The plates were then treated in the thermostat held at 58°C. for one hour. About ten hours later the germinated spores were removed to culture tubes, and those which had not germinated were treated for a second and sometimes for a third hour. Table 3 gives a record of the asci (from the 500 asci of *N. crassa*) in which ascospores were found that germinated without heating or that responded to heat-treatment of longer than one hour. Less than one per cent of the total number of spores germinated without heat-treatment; about four-fifths of the total number germinated following the first hour of treatment,

about two per cent of the spores yielded to the subsequent heat-treatments, and about one-fifth of the spores failed to germinate. The ratio in which the different types of spores occurred in certain asci, and their distribution in the ascus, suggest genetic differences, but these supposed differences have not been adequately tested.

TABLE 3

Fifty-two exceptional asci of Neurospora crassa from a total of over 500 studied. These are exceptional in containing some ascospores which did not respond to heat-treatment in the ordinary manner.

ASCOSPORE	1	2	3	4	5	6	7	8	ASCOSPORE	1	2	3	4	5	6	7	8
Ascus									*Ascus*								
217	1	1	1	1	2	2	x	2	642	1	2	x	1	3	3	2	1
222	1	x	1	1	1	2	1	1	643	1	1	1	1	1	2	1	1
257	1	1	1	1	1	1	2	1	644	2	2	2	2	x	x	2	2
286	1	0	0	1	x	1	1	1	646	2	x	1	1	1	1	2	1
312	1	1	x	2	x	x	x	1	650	1	2	1	2	1	x	1	1
322	x	2	1	1	x	1	1	1	651	2	2	2	2	1	1	1	2
330	1	1	1	1	0	0	0	0	654	2	1	1	1	1	1	1	1
332	1	2	x	2	2	2	x	2	655	1	2	2	2	1	1	1	1
336	2	1	2	2	1	1	x	2	657	1	1	1	1	1	1	1	2
340	x	1	1	2	1	1	1	2	659	1	1	1	1	2	1	1	1
341	3	2	1	2	1	1	1	1	676	1	1	1	1	1	1	2	1
342	ab	ab	ab	ab	1	2	2	2	677	1	2	1	1	1	2	1	1
361	1	1	x	1	0	0	x	x	678	1	2	1	1	1	1	1	2
498	x	1	1	1	0	x	0	1	683	x	1	2	1	1	1	1	1
500	1	1	1	1	1	1	0	1	688	1	2	1	1	1	1	1	1
550	1	1	2	2	2	1	1	1	715	2	2	1	x	1	1	1	1
551	2	2	x	x	1	1	1	1	718	1	2	1	1	1	1	1	1
552	1	2	2	2	2	2	1	1	724	1	1	1	1	1	2	1	2
553	1	1	1	1	2	2	2	2	725	1	1	1	1	1	1	2	1
554	1	1	1	1	2	2	2	2	730	1	1	1	1	1	1	1	2
555	1	1	2	2	1	1	1	1	731	1	1	1	1	1	2	2	1
556	1	1	2	2	2	2	2	1	732	1	1	1	2	1	1	1	1
557	1	1	2	1	1	1	1	1	741	2	3	2	2	3	1	2	2
562	1	2	1	x	1	1	1	1	744	1	2	2	2	3	1	1	1
563	2	x	1	0	x	0	2	2	745	2	3	3	x	x	x	2	2
641	2	2	3	3	1	x	3	3	690	1	1	1	1	2	2	2	2

Legend

1—spore germinated after first hour heat-treatment
2—spore germinated after second hour heat-treatment
3—spore germinated after third hour heat-treatment
0—spore germinated without heat-treatment
x—spore did not germinate
ab—spore aborted

In order to test this point selection experiments and subsequent crosses would have to be made. Only one experiment was performed. A mating was made between the mycelia from two of the ascospores that germinated without heating. Fifty-nine ripe ascospores were selected from the off-

spring. They were incubated on agar at 27°C. for nearly two days, but none germinated. A one hour heat-treatment induced fifty-five of them to germinate, indicating that they were mature. At first this was supposed to indicate that variations from response to one hour heat-treatment were not inherited. But subsequent experiments, with crosses of definitely in herited types, gave similar first generation results. Further generations, which in this case were not raised, would be required to prove this point.

It must also be noted that these asci were the inbred descendents from an ascus in which the spores germinated following a treatment of one hour, and it is possible that other races of *N. crassa* may exist whose spores require heat treatment for different lengths of time.

Nine asci of *N. sitophila*, described in table 4, showed a striking contrast to the case of *N. crassa*. It must be noted that all the asci of *N. sitophila* studied appear in table 4, while table 3 includes fifty-two aberrant asci selected from a total number of over 500. Therefore, in the case of *N. sitophila* about one half of the spores required two hours heat-treatment, while less than half of them germinated after one hour treatment.

TABLE 4

Nine asci of Neurospora sitophila showing the positions of the asocspores and the manner in which they responded to heat-treatment

ASCOSPORE	1	2	3	4	5	6	7	8	ASCOSPORE	1	2	3	4	5	6	7	8
Ascus									*Ascus*								
202	x	x	2	2	2	2	x	x	206	2	2	2	2	1	1	1	1
203	2	2	2	2	2	2	2	2	207	1	1	1	1	2	2	2	2
204	1	1	1	1	1	1	1	1	208	3	x	3	x	2	2	x	x
205	1	1	1	1	1	1	1	1	209	1	2	2	2	2	2	2	x
									210	1	2	1	2	1	2	1	1

Legend

1—spore germinated after first hour heat-treatment
2—spore germinated after second hour heat-treatment
3—spore germinated after third hour heat-treatment
x—spore did not germinate

In the case of asci 202, 206, and 207, the arrangement of the spores might suggest a segregation of a factor determining the response to heat-treatment, but no further breeding tests were made.

That the type of treatment necessary for germination depends on genetic factors is indicated clearly by the following experiments, which were started for another purpose, namely, to test the nature of a supposedly hybrid strain produced by Dodge (1931). By crossing a non-conidial mutant of *N. sitophila* with typical conidial *N. tetrasperma* and selecting and back crossing for several generations he had first secured a non-conidial

4-spored race. Then from this non-conidial, 4-spored type he grew a bisexual, hermaphroditic, non-conidial mycelium, which he designated 3C. He mated this with a unisexual, conidial mycelium of *N. tetrasperma*, producing thereby perithecia which were supposedly hybrid.

Proof that a hybrid had been produced between the hermaphroditic and the unisexual mycelium would be furnished if both conidial and non-conidial ascospores were found in the same ascus. The writer accordingly dissected sixteen exceptional 5-spored asci from this 4-spored hybrid. Since one parent was non-conidial and the other was conidial, 5-spored asci were selected in the expectation that, if a cross had been made, one of the two small unisexual spores from each of these asci would show the character of one parent while the other would show the character of the second parent. In each of the three other standard, bisexual, hermaphroditic spores, containing two genetically different nuclei, one from each parent, masking of the non-conidial character might be expected. As table 5 shows, all mycelia from the bisexual ascospores did produce conidia, indicating that the non-conidial character, if present, was masked. That the non-conidial character was actually present was shown by its manifestation in about half of the mycelia from the small spores. The presence of the conidial and the non-conidial nuclei proves that the supposed hybrid had actually been produced by the cross.

The data in table 5 are represented diagramatically in text figure 3. The 5-spored asci are grouped in classes according to the arrangement of the two small spores and the types of mycelia produced. The nuclei are shown as white, black, and black with a white center. The white nuclei indicate sex *A*; the black, sex *B*; the black nuclei with white centers were probably sex *B* but were incompatible with the tester strains used, so cannot be definitely determined in every case. Incompatibility is sterility with tester strains of opposite sex, a phenomenon resembling self sterility. The large, i.e., standard-sized, spores were assumed to be binucleate in origin, and the small spores uninucleate. Table 5 shows that the nuclei in most of the large spores contained two compatible nuclei, their sexes being indicated by $A+B$. A few of them carried incompatible nuclei, for the mycelia failed to produce perithecia. In such cases the sex is indicated by $A+B?$. The sign $+C$ indicates that the mycelium from the spore produced conidia, while $-C$ indicates that it was non-conidial. The mycelia from all of the large spores (except from two in ascus 408, discussed in detail below) produced conidia. Of the two small spores in each ascus one was conidial and the other was non-conidial (with the exception again of ascus 408, where both were $+C$). It is obvious from text figure 3 that the perithecia were true hybrids, since half of the nuclei in each ascus contained the conidial

TABLE 5

Ascospores dissected from fourteen asci obtained from hybrid perithecia referred to in the text. It is indicated whether or not the mycelia produced conidia, and whether or not the spores needed heat-treatment to induce germination.

SPORE	1	2	3	4	5
Ascus 402	L H + C A+B	L W + C A+B	S H + C B?	L H + C A+B?	S W − C A
Ascus 406	L H + C A+B	L H + C A+B	S H + C B?	L H + C A+B	S W − C A
Ascus 411	L H + C A+B	L H + C A+B	S H + C B?	L H + C A+B	S W − C A
Ascus 412	L W + C A+B	L H + C A+B	S H + C B?	L W + C A+B	S W − C A
Ascus 413	L H + C A+B	L NG	S H + C B?	L H + C A+B	S W − C A
Ascus 404	L H + C A+B	L H + C A+B	S H + C B	L H + C A+B	S W − C A
Ascus 410	L H + C A+B	L H + C A+B	S H + C B	L H + C A+B	S W − C A
Ascus 405	L W + C A+B	L W + C A+B	S W − C B?	L H + C A+B	S H + C A
Ascus 407	L H + C A+B	L H + C A+B	S W − C B?	L H + C A+B	S H + C A
Ascus 415	L H + C A+B	L H + C A+B	S W − C A	L H + C A+B	S H + C B?
Ascus 417	L H + C A+B	L H + C A+B	S W − C A	L H + C A+B?	S H + C B?
Ascus 403	L H + C A+B	L H + C A+B	L H + C A+B?	S W − C A	S H + C B?
Ascus 416	S W − C A	L H + C A+B	S H + C B	L H + C A+B	L H + C A+B
Ascus 408	L W − C A	L W − C A	S H + C B	L H + C B	S H + C B

Legend. *L*—Large, or standard sized, binucleate spore; *S*—Small spore, uninucleate; *H*—Spore needed heat-treatment to induce germination; *W*—spore germinated without heating; *+C*—Mycelium produced conidia; *−C*—Mycelium failed to produce conidia; *A* and *B*—Sex A or B; *A+B*—hermaphroditic, bisexual; *B?*—Sex B, incompatible with tester used; *A+B?*—hermaphroditic but producing no perithecia; *NG*—not germinated.

factor and half the non-conidial factor. Moreover, when a non-conidial and a conidial nucleus were present in the same ascospore, the mycelium produced conidia.

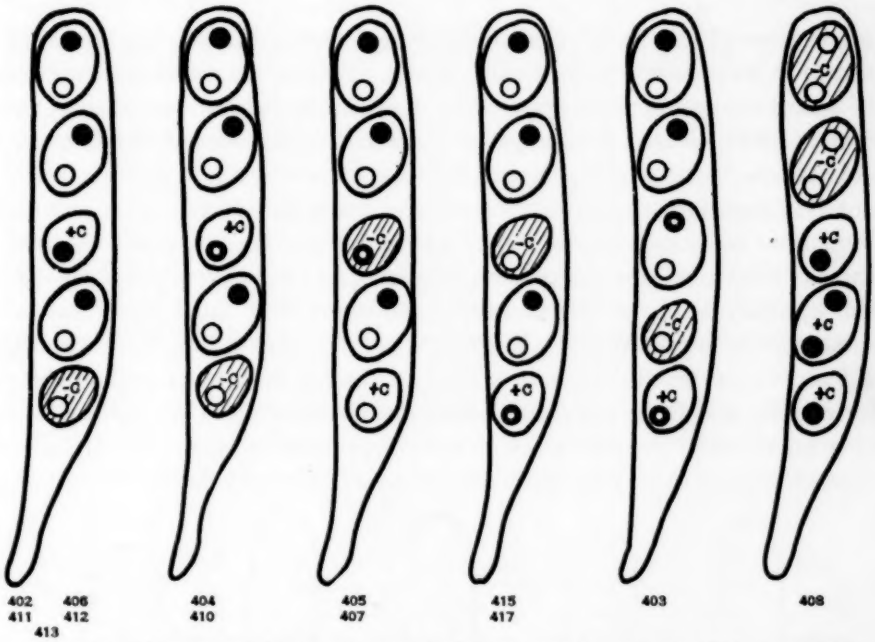

Fig. 3. Diagram of thirteen 5-spored asci dissected from a hybrid obtained by Dodge. The size, arrangement in the ascus, sex, and incompatibility of the spores are shown. The presence or absence of conidia on the mycelium and whether or not the spores require heat-treatment to induce germination are also shown. White nuclei are sex *A;* black nuclei are sex *B;* black nuclei with white centers are sex *B* incompatible; cross-lined spores germinate without heat-treatment; open spores require one hour heat-treatment; $-C$, the mycelium develops no conidia; $+C$, the mycelium develops normal conidia.

The special interest of this hybrid lay in the fact that Dodge had found that a large proportion of the spores germinated without heat-treatment. This characteristic appeared clear-cut in the spores dissected from the asci just described. In text figure 3, the diagonally lined spores are those which germinated without heating. The figure shows also that the spores which germinated without heating were all non-conidial. In all but two asci (405 and 407) these non-conidial spores that germinated without heating were moreover of sex *A*. This three-character linkage was very striking.

Ascus 408 was exceptional in two respects; first, the small spores were alike (B, $+C$, H), second, the three large spores were unisexual instead of hermaphroditic, and two were $-C$. This anomalous situation can be ac-

counted for in terms of the known peculiarities of the spindles leading to the formation of the spores, provided the segregation for these characters occurs at the second division. Dodge (1927) has described in detail the arrangement of the spindles in *N. tetrasperma*, showing that first-division segregation of factors for sex would always produce four hermaphroditic spores in each ascus. Since Dodge found no binucleate unisexual spores, he was of the opinion that segregation of sex factors always occurred at the first division, or that if segregation occurred at the second division, the orientation of the nuclei was such that unlike sexes came together. Without this peculiar orientation, second-division segregation would produce binucleate unisexual spores. Ascus 408 is of particular interest, since all three of the binucleate spores were unisexual, and hence presumably lacked this special orientation of the nuclei. Assuming that this hybrid has the same spindle mechanism as *N. tetrasperma*, we may conclude that ascus 408 shows second-division segregation of the sex factors as well as those for conidia and those for germinating without heating. Text figure 4 is a diagram showing the type of ascus behavior necessary to produce this rare kind of ascus. The straight lines are the axes of the spindles as they would

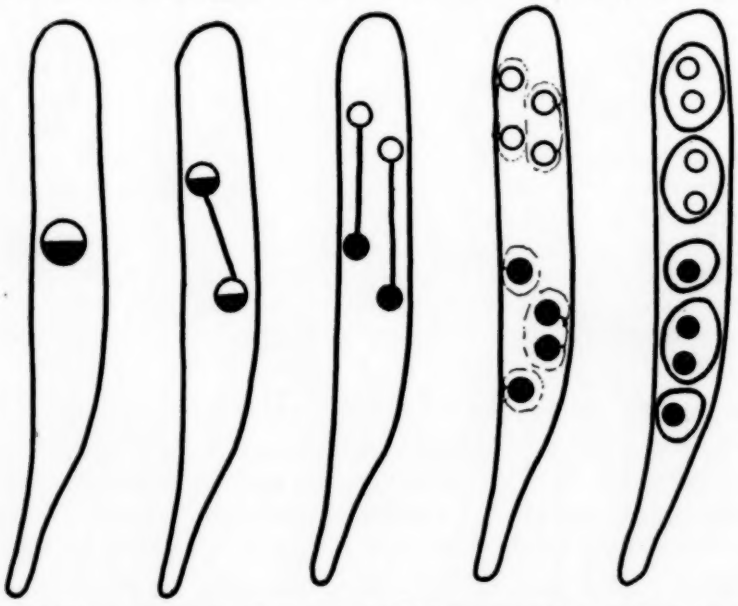

Fig. 4. Diagram showing how second-division segregation of the factors determining sex, response to heat-treatment and the presence or absence of conidia could give rise to a 5-spored ascus such as ascus 408, containing no hermaphroditic spores. White nuclei contain the factors for sex *A*, absence of conidia and ability to germinate without heat treatment. Black nuclei contain the factors determining sex *B*, production of conidia and inability to germinate without heat treatment.

lie if they followed the arrangements found for *N. tetrasperma*. This differs from Dodge's diagram for second-division segregation in only one feature, namely, that the orientation of one of the nuclei is reversed in the binucleate state of the ascus (text fig. 4).

The failure to produce perithecia in the case of the mycelia from spores from ascus 408 cannot be ascribed to incompatibility, since it was only sex *B* that showed incompatibility in this hybrid and in ascus 408 the sex *B* strain was tested and found compatible with the tester strain. That the failure to produce perithecia was actually incompatibility in the case of some of the mycelia of other asci was shown by the fact that the sex *B* strain from ascus 407, which failed to fruit with tester strains, fruited readily when mated with the sex *A* strain from ascus 406. This cross (406–5×407–3) was the mating of two mycelia both of which were non-conidial and were produced by ascospores which germinated without heating.

Twenty-one 4-, 5-, and 6-spored asci, from the cross 406–5 by 407–3, were dissected and the spores incubated at room temperature for several days. Table 6 shows the ascospores from this group which germinated

TABLE 6

Twenty-one asci dissected from a cross of two non-conidial ascospores that germinated without heating. The size and arrangement of the spores in the ascus are shown and whether or not they germinated without heating. Abbreviations as in table 5.

ASCOSPORES	1	2	3	4	5	6
Ascus 1	L H	L H	S H	L W	S W	
Ascus 3	S H	S W	L H	L H	L H	
Ascus 4	L W	S W	S H	L H	L W	
Ascus 5	L W	L W	S H	L H	S W	
Ascus 6	S W	L W	S H	L H	L H	
Ascus 7	L W	L H	S W	L H	S H	
Ascus 9	L H	L W	S W	L H	S H	
Ascus 10	L H	L H	S H	L H	S W	
Ascus 14	L H	L H	S W	L W	S H	
Ascus 15	L H	L H	S W	L H	S H	
Ascus 17	S H	S W	L H	L W	L H	
Ascus 18	L W	L W	S H	L W	S W	
Ascus 20	S W	L H	L H	L H	S W	
Ascus 16	S H	S H	S W	S W	L H	L W
Ascus 2	L W	L W	L W	L W		
Ascus 8	L W	L H	L H	L H		
Ascus 11	L H	L W	L H	L H		
Ascus 12	L H	L W	L W	L W		
Ascus 13	L H	L W	L W	L H		
Ascus 19	L W	L W	L W	L W		
Ascus 21	L W	L H	L W	S W		

without heat-treatment. Forty-five per cent of the large spores germinated without heating. Only twenty per cent of the ascospores in the parent hybrid germinated without heating. It is remarkable that in only one case did both small spores germinate without heating.

We may conclude from these experiments that the factor causing ascospores to germinate without heating is hereditary, and that it is usually carried by nuclei of sex *A*.

SUMMARY AND DISCUSSION

A microdissection apparatus was devised to rapidly remove single spores in order from the ascus in a genetic study of *Neurospora*.

Experiments were performed which show that it is possible to determine the optimum heat-treatment which will cause the largest percentage of the ascospores of *Neurospora* to germinate. Furthermore, it was shown that the heat-treatment commonly used killed the asexual spores, thus simplifying the separation of sexual from asexual offspring.

The ascospores from the race of *N. tetrasperma* seemed to be highly uniform in their response to heat-treatment. There was evidence that part of the small percentage of variation after one hour heat-treatment in *N. crassa* was due to genetic factors. Dodge's hybrid and the race of *N. sitophila* produced two types of ascospores in regard to their response to heat-treatment. In both races the differences were the result of genetic constitution, but there was evidence that the ability of a spore to respond to heat-treatment depended to some extent on the environment. This was best shown by the fact that although all of the large spores in the hybrid had one nucleus containing a factor for germinating without heat-treatment, and one containing a factor determining response to one hour heat-treatment, some germinated without heating while others did not. In Dodge's hybrid, the unisexual ascospores fell into two genetic groups: in one group all of the ascospores germinated without heat-treatment; in the other group the ascospores required one hour heat-treatment to initiate germination. The race of *N. sitophila* contained two genetic groups as well. One kind of ascospore required one hour heat-treatment, and the other required two.

Despite the evidence that response to heat-treatment is a genetic character in these cases, it is improbable that it can be used extensively in a study of the genetics of *Neurospora*, because of its variability. In a closely inbred race, such as the race of *N. crassa*, which seemed to be rather uniform in its response to a one-hour heat-treatment, ascospores were encountered which germinated without heating and others which required a three-hour treatment. Although in a few cases, the order of the spores in

the ascus indicated that factors determining response to heat-treatment had been segregated, it was obvious in most cases that the pairs of ascospores resulting from the third division in the ascus sometimes responded differently. It will be shown in later papers that this division is invariably equational. This means that these pairs of ascospores are identical genetically. The fact that they sometimes responded differently indicates that environmental factors were also effecting the variation.

Faull (1930) concluded, on the basis of some experiments on heat-treatment of the ascospores of a strain of *N. crassa*, that heat-treatment does not increase the percentage of germination. The data which she presents do not justify this conclusion. She found that from 4 to 25 per cent of the ascospores in various random samples germinated without heat-treatment. Ascospores in various random samples which she heat-treated at 51.5°C. for one to four hours showed 5 to 94 per cent germination. Obviously she was working with a mixed strain producing at least two genetically different kinds of ascospores.

The interest of Dr. T. H. Morgan in this problem has been a great encouragement. During the summer of 1930, the writer enjoyed the facilities of the New York Botanical Garden through the generosity of the director, Dr. E. D. Merrill. While there, he was closely associated with Dr. B. O. Dodge, and, through this association, received many valuable suggestions which have greatly facilitated the pursuit of the work. Dr. A. H. Sturtevant and Dr. Albert Tyler have offered many valuable suggestions. The writer is grateful to Dr. Morgan, Dr. Dodge, and Dr. Calvin B. Bridges for their assistance in revising the manuscript.

DEPARTMENT OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

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INDEX TO AMERICAN BOTANICAL LITERATURE

1927–1931

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Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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